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# THE NATIONAL MALARIA SOCIETY: PAST, PRESENT, AND FUTURE[1]

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Just a little over 51 years ago, in August 1897, Ross in India discovered the mechanism of the transmission of malaria. This permitted a sound epidemiological approach to the control of this devastating disease. The technics for antimosquito programs developed very promptly, including utilization of petroleum oil, drainage, filling, screening, et cetera. By the onset of World War I extensive malaria control projects had been initiated in the southern United States by the United States Public Health Service and the International Health Board, and the disease was recognized as preventable.

Boyd (1943) has discussed the early organization of the National Malaria Committee, the progenitor of our present National Malaria Society. Dr. Frederick L. Hoffman, the distinguished honorary chairman of the Committee for many years, successfully proposed the adoption of the following resolution before the Second Pan-American Scientific Congress, Washington, D. C., January 7, 1916:

> "That all American countries inaugurate a well considered plan of malaria eradication and control based upon the principle that the disease is preventable to a much greater degree than has thus far been achieved, and that the education of the public in the elementary facts of malaria is of the first order of importance to the country's concern."

Upon Hoffman's initiative a conference was held in Washington in May 1916, and the decision was reached to form a national committee on malaria whose objectives would be (a) to stimulate interest in malaria problems, (b) to serve as a medium through which societies and individuals may become identified with the study and prevention of the disease, and (c) to coordinate the efforts of these agencies with constituted Federal, state, and local authorities. Of 29 "charter" members listed by Boyd, at least 9 are still alive. The first general meeting of the Committee was held with the Southern Medical Association from November 12–15, 1917, at Memphis. It was decided at Memphis that future meetings would be in conjunction with those of the Southern Medical Association. Since malaria attains its highest level of endemicity in the United States in the southeastern section, this was a fortunate organizational arrangement.

## ANNUAL MEETINGS, MEMBERSHIP, AND CONSTITUTION

Boyd (1943) tabulated the dates and places of the first 25 annual meetings, together with the principal officers of the Committee. No meeting of the Southern

[1] Presidential address, meeting of the National Malaria Society, New Orleans, Louisiana, December 6–8, 1948.

Medical Association was held in 1918 because of the influenza epidemic. Table 1 brings these data current.

Table 2 has been kindly supplied by the secretary's office to which I am deeply indebted.

A few excerpts from past files, also supplied by the secretary's office, indicate that the formative years of the Society were quite informal:

"Neither the Honorary nor the Acting President being present, the meeting was called to order at 9 o'clock by the Secretary, H. R. Carter, Assistant Surgeon General, U.S.P.H.S. After the invocation an address of welcome was delivered by Dr. Andrews, the Health Officer of Memphis, the key-note of which seemed to be that malaria was absent from that city.

"The session was declared by the Secretary to be an open session of the Committee in which papers would be read upon subjects connected with malaria . . . and these papers would be open to debate. He apologized for and explained his inability to assign men to open these different debates on the ground that he had been engaged

TABLE 1

*Annual Meetings*

| YEAR | DATES | PLACE | PRESIDENT | SECRETARY |
|---|---|---|---|---|
| 26th | 1943 | Cincinnati, O. | J. S. Simmons | M. F. Boyd |
| 27th | 1944 | St. Louis, Mo. | G. H. Bradley | M. F. Boyd |
| 28th | 1945 | Cincinnati, O. | H. A. Johnson | M. F. Boyd |
| 29th | 1946 | Miami, Fla. | M. F. Boyd | M. D. Young |
| 30th | 1947* | Atlanta, Ga. | M. D. Hollis | M. D. Young |
| 31st | 1948* | New Orleans, La. | E. H. Hinman | M. D. Young |

* Met independently of the Southern Medical Association but conjointly with the American Society of Tropical Medicine and the American Academy of Tropical Medicine.

in field work on the environment of cantonments up to within 5 days of the opening of the meeting and had had no time to do so."

Transmitting the minutes of that session for the President (Rupert Blue, Surgeon General at that time), Dr. Carter made the following comment on the meeting:

"The meeting was a success, I think a rather brilliant success, only too many and too small new men were admitted. That must be provided against in the future . . . . One other error, for which I am responsible, is that Mr. Hoffman's resolution report of the committee on statistics should have stopped at the end of the second paragraph, but I had amended that paragraph, and was almost dead for sleep, having been up all night the night before, and did not notice the futility of the fourth and fifth paragraphs."

Speaking of the meeting of the Executive Committee on June 5, 1919, the Secretary said, "This meeting was a failure—well nigh a complete failure."

At the general meeting November 1919 there were so few speakers present that the morning session had to be postponed and the afternoon session substituted. That same evening of the first day's session, the Executive Committee was forced to continue the same officers in office for the forthcoming year inasmuch as there

TABLE 2

*Meetings and Membership of the National Malaria Society*

| NO. | YEAR | DATES | PLACE | REMARKS | NO. OF MEMBERS |
|---|---|---|---|---|---|
| | 1916 | May 10 | Washington, D. C. | 13 workers met to found the National Malaria Committee. Secretary elected. | 29 (charter) |
| | 1916 | Nov. | Atlanta, Ga. | Chairman and secretary elected. | Minutes not available |
| | 1917 | June 6 | New York, N. Y. | Executive Committee met. | Minutes not available |
| 1 | 1917 | Nov. 12–13 | Memphis, Tenn. | First general meeting; met conjointly with SMA; "more than 75 attended." | 38 |
| | 1918 | — | — | No meeting because of influenza epidemic. | |
| | 1919 | June 5 | Washington, D. C. | Executive Committee met. Membership need not be a prerequisite to appointment on subcommittee. | 41 |
| 2 | 1919 | Nov. 10–12 | Asheville, N. C. | Met conjointly with SMA. Approx. 75 persons present. | 43 |
| 3 | 1920 | Nov. 15 | Louisville, Ky. | Met conjointly with SMA. | 31 |
| 4 | 1921 | Nov. 14 | Hot Springs, Ark. | Met conjointly with SMA. | 58 |
| 5 | 1922 | Nov. 13 | Chattanooga, Tenn. | Met with SMA. | 69 |
| 6 | 1923 | Nov. 12–13 | Washington, D. C. | Met with SMA. Only 21 members were present. | 73 |
| 7 | 1924 | Nov. 24 | New Orleans, La. | Met with SMA. | 81 |
| 8 | 1925 | Nov. 10–12 | Dallas, Tex. | Met with SMA. | 108 |
| 9 | 1926 | Nov. 17–18 | Atlanta, Ga. | Met with SMA. | 120 |
| 10 | 1927 | Nov. 16 | Memphis, Tenn. | Met with SMA. | 134 |
| 11 | 1928 | Nov. 14–15 | Asheville, N. C. | Met with SMA. | 158 |
| 12 | 1929 | Nov. 21–22 | Miami, Fla. | Met with SMA. | 165 |
| 13 | 1930 | Nov. 11–14 | Louisville, Ky. | Met with SMA. Adopted new constitution; annual dues ($1). | 175 |
| 14 | 1931 | Nov. 18–20 | New Orleans, La. | Met with Amer. Soc. of Trop. Med. in one conjoint session; met with SMA. | 203 |
| 15 | 1932 | Nov. 16–18 | Birmingham, Ala. | Met with ASTM and SMA. Revised membership to include active and honorary. | 205 active; 19 hon. |
| 16 | 1933 | Nov. 15–17 | Richmond, Va. | Met with SMA and ASTM. | 167 active; 17 hon. |
| 17 | 1934 | Nov. 14–16 | San Antonio, Tex. | Met with ASTM and SMA. | 151 active; 18 hon. |
| 18 | 1935 | Nov. 20–22 | St. Louis, Mo. | Met with ASTM and SMA; dues changed to $2 to defray cost of reprints of papers. | 148 active; 19 hon. |
| 19 | 1936 | Nov. 19–20 | Baltimore, Md. | Met with ASTM and SMA. | 148 active; 18 hon. |
| 20 | 1937 | Dec. 1–3 | New Orleans, La. | Met with ASTM and SMA. | 168 active; 18 hon. |
| 21 | 1938 | Nov. 16–18 | Oklahoma City, Okla. | Met with ASTM and SMA. | 173 active; 19 hon. |
| 22 | 1939 | Nov. 22–24 | Memphis, Tenn. | Met with ASTM and SMA; *Malaria Control for Engineers* published in cooperation with American Society of Civil Engineers. | 198 active; 20 hon. |
| 23 | 1940 | Nov. 13–15 | Louisville, Ky. | Met with ASTM and SMA; first affiliated with Amer. Assn. Advancement of Science (Jan. 22). | 190 active; 20 hon. |
| 24 | 1941 | Nov. 11–13 | St. Louis, Mo. | Met with ASTM and SMA; dues raised to $3 and name changed to Nat. Mal. Soc. Publication of Journal authorized. | 184 active; 20 hon |
| 25 | 1942 | Nov. 10–12 | Richmond, Va. | Met with ASTM and SMA; Jour. appeared as annual. | 187 active; 20 hon. |
| 26 | 1943 | Nov. 16–18 | Cincinnati, O. | Met with ASTM and SMA; Jour. issued as semiannual. | 274 active; 19 hon. |
| 27 | 1944 | Nov. 14–16 | St. Louis, Mo. | Met with ASTM and SMA; Jour. issued as quarterly. | 394 active; 21 hon. |
| 28 | 1945 | Nov. 13–14 | Cincinnati, O. | Met with ASTM and SMA. | 424 active; 21 hon. |
| 29 | 1946 | Nov. 5–7 | Miami, Fla. | Met with ASTM and SMA; new constitution adopted vesting government in Board of Directors; honorary membership discontinued. | 504 active; 19 hon. |
| 30 | 1947 | Dec. 2–4 | Atlanta, Ga. | Met with ASTM; first general meeting without SMA; constitution amended; dues raised to $4. | 554 |
| 31 | 1948 | Dec. 6–8 | New Orleans, La. | Met with ASTM and ASP. | 572 |

were not enough present to hold an election (at least a representative election). The following morning there was no quorum present at the meeting, and the business which was to have been considered was later transacted by correspondence.

Originally the National Malaria Committee was considered as the Malaria Division or symposium of the Section on Public Health of the Southern Medical Association, and the latter organization permitted its Malaria Commission which had been formed in 1912 to lapse. A constitution and set of bylaws were adopted at the Louisville meeting which provided a more adequate organizational pattern. In 1941 the name was changed to the National Malaria Society. A completely new constitution was

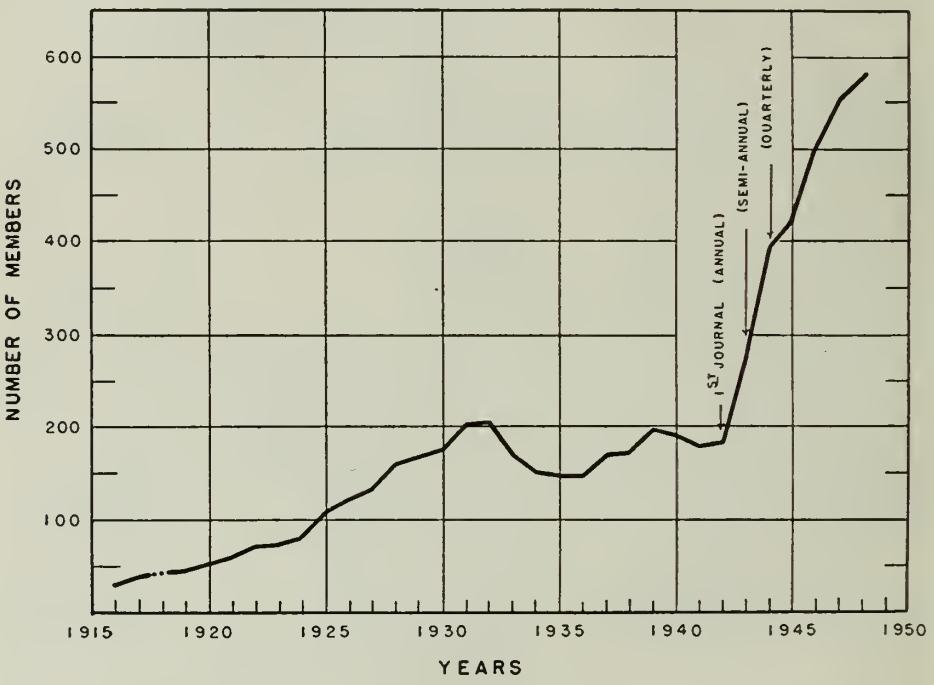


Fig. 1. Active membership of National Malaria Society

adopted by the Society at the Miami meeting and has been published in the *Journal of the National Malaria Society*, 6: 87–90, 1947.

The National Malaria Committee and its successor, the National Malaria Society, enjoyed a most cordial association with the Southern Medical Association through its 29th annual meeting in Miami, Florida, in 1946. As the host organization grew and with increasing difficulty in obtaining hotel and convention facilities for annual meetings, the Southern Medical Association reluctantly decided that conjoint meetings were no longer possible. Our Society is happy to acknowledge its indebtedness to its generous host. With the Atlanta meeting it may be said that the National Malaria Society "came of age" and entered upon a new era. An official registration of 132 members demonstrated the ability of the Society to stand on its own feet (a far cry from the 1919 meeting when no quorum was present). The Atlanta meeting

was held conjointly with the American Society of Tropical Medicine and the American Academy of Tropical Medicine. The current meeting involves a similar arrangement except that the American Society of Parasitologists has been invited to participate.

Starting with an original membership of 29 charter members, the National Malaria Committee grew slowly and steadily until in 1941, the membership roster had reached 204. Today we are proud to report that there are 572 active members. Of the original 29 charter members the following are still living:

C. C. Bass
C. F. Craig
S. Harris
L. O. Howard
Arthur Hunter
J. A. LePrince
J. W. King
W. S. Rankin
J. M. Swan

Figure 1 presents our growth curve. The most striking growth has occurred since 1941, when the name of the organization was changed to the National Malaria Society. It is believed that the increase since 1941 is closely associated with the initiation of the official journal of the Society. The occurrence of World War II accentuated interest in malaria, but without its official publication the Society would not have been in a position to render its maximum contribution.

## PUBLICATIONS

From 1921 to 1941, with the exception of 1933, the Southern Medical Association published papers from the National Malaria Committee, together with certain subcommittee reports, in the *Southern Medical Journal.* The Rockefeller Foundation provided reprints of these for distribution to members from 1925 to 1929, and the Southern Medical Association donated reprints of the symposia to members from 1930 to 1932. The National Malaria Committee purchased reprints for distribution to its members from 1934 to 1941.

In 1942 the National Malaria Society published the papers which had been presented at the 1941 meeting as Volume 1 of the *Journal of the National Malaria Society.* This was a single number, in 1943 the journal appeared as a semi-annual, but since 1944 it has appeared as a quarterly publication. Originally the journal published only the proceedings of the annual meeting, but it quickly began the practice of accepting contributed papers.

The editorial board has been composed of those shown in table 3.

The accompanying table indicates the growth of the journal which has been supported by two-thirds of the $3.00 annual dues of the Society through 1947 and the sale of a limited amount of advertising space. In 1948 the membership dues were increased to $4.00 of which $3.00 is placed in the publication fund.

During 1948 the Society was most fortunate in obtaining a grant of $1,000 from the American Foundation for Tropical Medicine to support and extend the scope of the journal. With increasing costs of publication, this is most timely and will

permit the publication of a number of acceptable manuscripts which it would not otherwise be possible to print. This mark of recognition of the standing of our journal and desire to support it is most welcome, and it is desired on behalf of the Society to express publicly our gratitude. The editorial board has under consideration the proposal to issue the journal as a bimonthly publication beginning in 1949.

The journal is unquestionably the most productive activity of the National Malaria Society. In convassing for additional membership, it is the most tangible asset which can be presented to the prospective member. In one additional way may the membership contribute to the support of the journal and thereby to that of the Society. A substantial source of revenue for its publication is derived from its advertisers. If the membership could assist in enlisting more advertisers, our journal could be strengthened. The editor, the business manager of the journal, and their associates seek your cooperation in this matter.

TABLE 3

| VOLUME | YEAR | PAGES OF JOURNAL | EDITOR | OTHER MEMBERS OF EDITORIAL BOARD |
|---|---|---|---|---|
| 1 | 1942 | 182* | C. F. Craig | J. M. Andrews, N. H. Rector, and M. F. Boyd |
| 2 | 1943 | 220† | C. F. Craig | J. M. Andrews, L. G. Lenert, and M. F. Boyd |
| 3 | 1944 | 273 | C. F. Craig | J. M. Andrews, L. G. Lenert, and M. F. Boyd |
| 4 | 1945 | 364 | R. B. Watson | L. G. Lenert and R. L. Usinger |
| 5 | 1946 | 284 | F. L. Knowles | R. L. Usinger, N. H. Rector, and M. D. Young |
| 6 | 1947 | 252 | F. L. Knowles | N. H. Rector and E. H. Hinman |
| 7 | 1948 | 384 | F. L. Knowles | N. H. Rector and E. H. Hinman |

* 1 number.

† 2 numbers plus supplement.

## SPECIAL ACTIVITIES

It is desired to call your attention to certain special activities of the National Malaria Society or Committee or of its membership in the furtherance of its objectives. In 1937 Dr. L. L. Williams and others of the Malaria Investigations Unit of the U. S. Public Health Service induced most of the State Health Departments of the southeastern states to set up within their organizations a separate malaria unit which ideally would be composed of a medical malariologist, a malaria engineer, and an entomologist. The training of these individuals was provided by the Savannah Laboratory of the U. S. Public Health Service, the Orlando Laboratory of the Bureau of Entomology and Plant Quarantine, the Tallahassee Malaria Station of the Rockefeller Foundation, and the Malaria Studies and Control Division of the Tennessee Valley Authority. Since funds were available under public works programs for permanent elimination of anopheline mosquito breeding places, this was a most timely activity and no doubt made its contribution toward the decline in malaria rates which began to occur a short time thereafter.

The engineering membership of our Society has always been most active. A teaching syllabus, "Malaria Control for Engineers," was first mimeographed by the U. S. Public Health Service and later published in the *Proceedings of the American Society of Civil Engineers*, 1939. A variety of special engineering reports have appeared before and since the above-mentioned publication. The latest was a reference code for use in the recording, estimating, and reporting of physical accomplishments and costs in mosquito control operations, prepared by Messrs. Henderson, Dorer, Kiker, Lee, and Van Hovenberg, and published in the June 1948 number of the Journal.

Special committee reports on epidemiology, vital statistics, medical research, entomology, et cetera have appeared in the annual symposia and elsewhere.

In the spring of 1940 with the darkening of war clouds in Europe the Surgeon General of the U. S. Public Health Service called a conference at Atlanta, Georgia, to consider some of the fundamental aspects of malaria research and of exploring opportunities for stimulating its extension and coordination. The majority of the participants in this conference were members of the National Malaria Committee. The report of this conference was published in Volume 55 of the Public Health Reports, pages 1801–1809, October 4, 1940.

In 1940 the annual meeting of the American Association for the Advancement of Science sponsored a "Symposium on Human Malaria." The National Malaria Committee was one of the sponsoring organizations, and the organizing committee was drawn from our membership. The publication of the "Symposium on Human Malaria" by the AAAS just prior to the United States' entrance into World War II was a major contribution toward national preparedness. Of its 42 contributors all but 4 were members of the National Malaria Committee.

At the 1942 annual meeting of the National Malaria Society the president of the Society was empowered to appoint a committee consisting of a physician, a sanitary engineer, and an entomologist to cooperate with the Army, the Navy, and the U. S. Public Health Service and to make available to them the technical skill and knowledge possessed by the members of the National Malaria Society. Elsewhere there is summarized briefly some of the contributions of the membership to the war effort.

At the 1943 annual meeting in Cincinnati a resolution was adopted that the National Malaria Society endorse the program proposed by Dr. Joseph Mountin, of the U. S. Public Health Service, for the final eradication of malaria from the continental United States.

The Malaria Control in War Areas program was a joint undertaking by the U. S. Public Health Service and State Health Departments, designed to reduce the hazard of malaria transmission in extra-cantonment zones of military areas and around essential war industries. It complemented similar activities of military authorities within reservation limits. This program was subsequently extended at the close of the war in order to prevent the secondary transmission of malaria from veterans returning to civilian life from highly malarious areas. Subsequently, the program of the Malaria Control in War Areas was absorbed by the Communicable Disease Center.

The Society was one of the sponsors of the Fourth International Congresses of

Tropical Medicine and Malaria in Washington May 10–18, 1948. Your president was privileged to serve as one of the official United States delegates to the Congresses and subsequently was elected a member of the Interim Committee for the Fifth Congress. The section on malaria held six scientific sessions at which 38 papers were presented. In addition, all malariologists in attendance at the Congresses were invited by the Interim Committee of the World Health Organization, Expert Committee on Malaria, to attend a special meeting of the committee at which opportunity was provided to suggest ways in which the World Health Organization might further their objectives in the field of malaria control.

TABLE 4

*Contributions of Membership of the National Malaria Society to World War II*

(Based upon 245 replies to questionnaire)

| | NONE | ARMY | NAVY | USPHS | FOREIGN SERVICE |
|---|---|---|---|---|---|
| Active Military Service | 86 | 75 | 18 | 66 | 91 |
| | FEDERAL | | OTHER | | |
| Special Civilian Employment | 19 | | 45 | | |
| | MILITARY | | CIVILIAN | | |
| Consultant Appointment (Secretary of War, NRC, etc.) | 38 | | 24 | | |
| | TEACHING | | RESEARCH | | |
| Teaching or Research (Related to war effort) | 50 | | 47 | | |
| | MILITARY | | CIVILIAN | | |
| Decorations Awarded (Other than theater or campaign ribbons) | 81 decorations to 47 individuals | | 19 decorations to 11 individuals | | |

## WAR SERVICE OF MEMBERSHIP

The Society is justly proud of the contributions which its membership made to the war effort during World War II, both in the military and civilian fields. It has not been possible to obtain replies from the entire membership to a questionnaire sent out concerning war service. Of 245 individuals replying to the questionnaire, 159 members were in active duty on a military status; of these 75 were in the Army, 18 in the Navy, and 66 in the U. S. Public Health Service, with 91 being on foreign duty. Two members, Lt. William Gordon, U. S. N., and Major Roy E. King, A. U. S., died while on active duty. Those not participating through active military duty were, to a large extent, involved in the war effort through essential civilian appointments, table 4. Recognition of important contributions was indicated by the comparatively large number of our membership receiving either military or civilian decorations.

## POSTWAR TRANSITION

With the millions of men returning from active duty in malarious areas of the tropics, there was serious apprehension that malaria in the United States, which had been steadily declining, might flare up in a widespread manner throughout a great portion of the United States, and it was for this reason that the extended program of MCWA was undertaken. Despite numerous relapses among veterans in areas with substantial anophelism, fortunately, these fears were unfounded.

Andrews (1948) referred to the general decline of malaria in this country which was believed to have begun during the last quarter of the nineteenth century. While its retreat was interrupted by resurgences in prevalence on a 5-year to 7-year cyclical occurrence, since the mid-1930's the decline has been abrupt and continued.

At the Miami meeting in 1946 preliminary discussion was held concerning the possibility of broadening the base of interest of the membership of the Society. With the decline in malaria rates and the cessation of hostilities the emphasis on malaria as a public health problem in the United States was certain to decrease. The close cooperation of physicians (epidemiologists), public health engineers, and biologists had proven a useful technique in the attack upon malaria and was being employed in other public health offensives against such diseases as endemic typhus, fly-borne enteric infections, et cetera. In fact, the same personnel in many instances were being utilized to combat malaria and other arthropod-borne diseases. It was felt that our Society was in a position to assist in public health campaigns other than malaria and, therefore, we should extend the range of our interests. No definitive action was taken, but at the 1947 meeting the incoming president was directed to appoint a committee to study this matter. The committee has consisted of Dr. Paul F. Russell, chairman, Dr. Justin Andrews, Dr. E. C. Faust, and Mr. John H. O'Neill, with the secretary-treasurer and the president members ex officio.

In appointing the committee, your president wrote in part as follows to Dr. Paul F. Russell on December 12, 1947:

"It is becoming evident that malaria as a public health problem within the continental United States may disappear largely within the next few years. This, of course, does not indicate that the United States will lose any of its concern in the control of malaria, for it will have to maintain a program of surveillance and emergency control within its continental borders, and it is not likely that within such a brief period the interest in the control of malaria will diminish greatly in many of the tropical countries of the world in which the United States has vital interests. In view of the homogeneity of interests of our membership, which is quite unusual in view of the heterogeneity of their professional background, it is believed that it would be most undesirable to countenance the possibility that our membership might disintegrate and that no adequate provision be made for retaining the professional contacts which have been so useful in the past.

"Another consideration is that at the moment many of our members are engaged in public health control programs of arthropod-borne diseases. The techniques of these programs, the professional interests of the workers, are in many ways similar to those which have been developed for the control of malaria. This is particularly true in the case of typhus control, Rocky Mountain spotted fever, plague, the control

of flies which may be vectors in the transmission of dysenteries and, possibly, even poliomyelitis. It is my feeling that the sentiment which has been expressed during the last year represents genuine feeling on the part of certain of our membership that the base of interest of the Society might appropriately be broadened to include certain arthropod-borne diseases.

"In thinking of this matter, it seems to me that there are several possibilities, and I am sure that you and other members of the committee will add to such a list. The motion presented to the Society was merely that the base of interest of the National Malaria Society might be broadened to include arthropod-borne diseases. We might consider this merely one trend. A second possibility which might be considered is actual fusion with such an organization as the American Society of Tropical Medicine. A third possibility would be to reorganize under a totally different name or to fuse with some other organization having certain more or less parallel interests. A rather drastic suggestion which has occurred to me is that the Society consider the appropriateness at some time in the future of disbanding on the basis that it has accomplished the objectives for which it was organized, the basis for this being that if malaria were successfully eradicated from the United States at some time in the not-too-distant future the organization could claim the unique distinction of having attained its objectives and being ready to dissolve by mutual agreement. If so, such dissolution should occur only after the eradication of malaria in the United States has become an established fact."

The committee has been very active by both correspondence and direct contact as opportunity has afforded, and it will report to the Society at its annual meeting in some detail, although the membership has already been advised of the thinking of the committee members in part as follows:

"In recognition of the broadening of interest of the membership of the National Malaria Society consideration should be given at the next annual meeting to a change in name of the Society which would be in keeping with the interests of this membership. It is not proposed by the committee that definitive action be taken at this forthcoming annual meeting but rather that opportunity be afforded for suggestions to be proposed by the membership and full discussion prior to any revision of the constitution. Your cooperation in this matter is requested, and suggestions should be mailed to Dr. Paul F. Russell, Laboratories of the International Health Division, Rockefeller Institute for Medical Research, York Avenue and Sixty-sixth Street, New York 21, N. Y. Certain alternate names which have been suggested include: 'National Society for the Study and Control of Arthropod-Borne Diseases'; 'National Society of Malaria and Other Arthropod-Borne Disease'; and 'National Society of Arthropod-Borne Disease.' It is hoped that briefer names may be acceptable.

"The committee believed that it would be profitable if the rather informal association between the American Society of Tropical Medicine, the American Academy of Tropical Medicine, and the National Malaria Society might be formalized, looking toward the establishment of a Federation which might be called, for example, the 'American Federation of Societies of Tropical Diseases and Sanitation' or 'American Federated Societies of Malaria and Tropical Diseases.' Such a Federation might include other organizations than those referred to above. If the trends of interest

of the members of the National Malaria Society were broadening in scope, this matter should be discussed with our sister societies with whom consideration is being given for federation in order that there not be duplication or overlapping of interests. It, therefore, would seem appropriate that the National Malaria Society assume the initiative in accordance with the suggestion of Dr. George K. Strode in his presidential address to the American Academy of Tropical Medicine. In looking toward a closer integration of the activities of the three organizations, it is hoped that a draft of a loose federation may be presented at the New Orleans meeting which will indicate the ways in which the executive officers of the three societies would coordinate their activities, particularly with reference to meeting place, arrangement of program, joint meeting, et cetera. Actually this does not represent any closer union than probably already exists but would place on record a procedure for its accomplishment. It is possible that this relationship might be subsequently strengthened to the point of coalition."

An interesting development in connection with the Fourth International Congresses of Tropical Medicine and Malaria recently held in Washington was the decision to fuse permanently the two Congresses but not to permit malaria to drop to the position of an ordinary section under the Congress. In order to prevent this, provision was made that the officers would always include one vice president from Tropical Medicine and one from Malaria. This precedent was also established in the election of the Interim Committee to plan for the fifth united Congress. It is quite possible that these Congresses have established a pattern which our national societies might follow.

## THE FUTURE

Now that a brief review has been presented of the past and present activities of our Society, it is time to look into the future. Russell (1948) has listed six very specific reasons for insisting upon the continuing importance of malaria to the United States. National defense plans must contemplate the possibility of coping with malarious areas; the acute world food shortage is intensified by areas undeveloped largely because of malaria; American business is expanding into malarious countries and is concerned with the efficiency of malaria control; all our imports from malarious countries carry a hidden "malaria tax"; our exports to malarious countries are restricted because malaria depresses the economic levels; and finally there is a need to prevent the resurgence of malaria in our own country.

Andrews (1948) points out that since socioeconomic progress has undoubtedly stimulated the development of most of the factors responsible for the decline in malaria in the southeastern United States then a depression might permit malaria to become again a public health hazard of great prominence.

In view of its notable achievements in the past, the question may be asked as to how the Society may make its maximum contribution in the future. The development of its journal should receive primary attention, for the benefit from this activity is not confined to the membership of the Society, but is and should continue to be a potent tool in the waging of war against malaria the world over.

From an organizational point of view our Society is at the crossroads. Your

Committee on Policy has recommended consideration of broadening the base of interest of its membership. This would preserve the identity of our organization and extend its influence into other related fields. The association of physicians, engineers, and biologists, as pointed out elsewhere, has been productive in the control of malaria, and the techniques developed by these combined professions can be applied with undoubted success to the control of many arthropod-borne diseases. A danger must be recognized in the possibility of infringing upon the interests of other professional societies. A second alternative which has been considered by the committee would be coalition of the National Malaria Society with one or more other organizations having similar interests, as for example, the American Society of Tropical Medicine. Already many individuals are members of both societies (191 of our 572 members). The combination of the journals of the two organizations should result in a really effective monthly publication and reduce the total cost of membership to the duplicating members. From the point of view of the National Malaria Society such a fusion would not be totally satisfactory unless there was provision for parity of the medical, biological, and engineering professions from the standpoint of sharing the responsibilities of office-holding, policy determination, et cetera. At the moment it would not be possible to forecast the probable reaction of the American Society of Tropical Medicine or any other professional society to such a proposed coalition. This alternative might be looked upon as one for the future, and if a federation is achieved then the latter may be step one in attaining this objective. A third possibility is for the National Malaria Society to retain its present constitution and work steadily toward the achievement of its objectives, namely, to advance "knowledge regarding the cause, prevalence, epidemiology, treatment, prevention, and control of malaria through integration of activities of the various specialized fields of endeavor and through stimulation of scientific and practical interest among organizations and individuals in the prompt and effective application of treatment and control methods." In this eventuality it is possible that if malaria were successfully eradicated from the United States the Society might wish to dissolve, since it would be in the unique position of having attained its objectives.

The decision in these matters rests with the membership. Your officers, members of the Board of Directors, and committee members wish to elicit your opinions and from your desires formulate a policy which can be executed in accordance with these wishes.

One additional problem is called to the attention of the membership of the National Malaria Society gathered in attendance at its thirty-first annual meeting, namely, the propriety of establishing an annual lectureship or the award of a medal commemorating the outstanding achievements of a pioneer in the field of malariology. A personal preference is expressed for the recognition of an American scientist who may have been active in the organization of our Society. The names of workers such as Carter, LePrince, and others immediately suggest themselves. The American Society of Tropical Medicine has already appropriated the name of Charles Franklin Craig for its annual lectureship. It is proposed that the incoming president be instructed to appoint a committee of three to examine this recommendation and

report to the Board of Directors within a period of 6 months in order that any definitive action may become effective prior to our next annual meeting.

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# PHYSIOLOGICAL STUDIES IN THE HUMAN MALARIAL HOST

## I. Blood, Plasma, "Extracellular" Fluid Volumes and Ionic Balance in Therapeutic *P. vivax* and *P. falciparum* Infections[1,2]

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Although numerous integrated studies of the life cycle, biochemistry, metabolism, and susceptibility to drugs have been made on the various plasmodia which infect man and laboratory animals, there are few such correlated studies of the malarial host. Published observations of alterations in physiological and biochemical response of the malarial host have been isolated and consist largely of studies of one or a few physiological variables. These observations have been briefly reviewed by Maegraith (1948). Studies of blood volume, plasma volume and plasma protein concentration in human therapeutic malaria were made by Feldman and Murphy (1945), although only 9 patients were studied and but few variables reported.

The present studies involve repeated measurements in the same patients of blood, plasma, "available fluid", and erythrocyte volumes, serial determinations of Na, K, and Cl in whole blood, plasma, erythrocytes and urine. Likewise, 24-hour urinary volumes, 24-hour excretions of Na, K, and Cl, alterations in body weight, plasma protein concentrations, total circulating protein, parasitemia, hematocrit alterations and body temperature changes were followed in 70 patients with central nervous system syphilis, 42 of whom were therapeutically infected with *P. vivax* (McCoy strain) and 28 with *P. falciparum* (Costa strain). The division between those receiving mosquito-borne disease and those infected by direct blood transfer of trophozoites was roughly equal.

### MATERIALS AND METHODS

Prior to infection with malaria, control determinations of the variables previously listed were made as follows: (1) plasma volume was measured (in fasting subjects) by the T-1824 dye dilution method (Gibson and Evans, 1937). To circumvent the possibility that early dye disappearance or sequestration might be abnormal in these patients, the multiple (5) sample technique was used rather than the single sample

[1] The work described in this paper was supported by grants-in-aid from the U. S. Public Health Service, Division of Research Grants and Fellowships.

[2] A portion of the data presented in this paper appeared as part of an exhibit at the Fourth International Congresses on Tropical Medicine and Malaria, May 10–18, 1948 at Washington, D. C.

[3] The authors gratefully acknowledge the technical assistance of Mr. A. K. Davis and Mrs. Anne C. Bass.

method employed by Feldman and Murphy (1945).[4, 5] The absorption coefficient of the dyed samples of plasma was determined with a Coleman photoelectric spectrophotometer, (2) hematocrit determinations were made using Wintrobe tubes which were centrifuged for 45 minutes at 2300 G., (3) total blood volumes and erythrocyte mass were calculated from the plasma volume and hematocrit, (4) the volume of fluid available for the dilution of thiocyanate (SCN) ("extracellular fluid volume") was determined by a modification of the method of Crandall and Anderson (1934). Ten cubic centimeters of a 5 per cent solution of NaSCN was intravenously injected and SCN determinations made on each plasma sample subsequently drawn as suggested by Gregersen and Stewart (1939), (5) plasma and whole blood specific gravities were measured by the copper sulfate method of Phillips, et al. (1943). Plasma protein concentration was estimated from plasma specific gravity and the total circulating protein was calculated from this concentration and the measured plasma volume. (6) Simultaneously with the measurements previously described, 10 cc. of heparinized venous blood was obtained for ionic studies. Sodium and potassium concentrations were measured in whole blood, plasma, and packed erythrocytes by the flame photometric methods of Overman and Davis (1947). Similarly, an aliquot of the 24-hour pooled urine for the preceding day was analyzed. (7) Chloride determinations on blood, plasma, red cells and urine were made using a modification of the method of Volhard (Peters and Van Slyke, 1932).

Following the establishment of control values, each patient was infected with malaria and serial determinations were made throughout the active clinical phases of the disease and during convalescence, which followed chemotherapeutic intervention with atabrine, quinine, plasmochin, paludrine, or chloroquine. On the day of each determination 24-hour urine volume, body weight, blood pressure, heart rate, body temperature, and parasitemia were recorded.

## RESULTS

Since more than 250 determinations of the 25 variables were made in this study, and since we are dealing with both *P. vivax* and *P. falciparum* infections, numerous methods of presentation of the data have been assayed. In general, the results to be presented qualitatively parallel those previously reported in *P. knowlesi* infections in monkeys (Overman and Feldman, 1947) (Overman, 1946) (Overman, 1948). However, since the absolute parasitemic levels of the two diseases are quite different, the combined data could not easily be correlated with *this* variable. Repeated analyses of the data show that the physiological and biochemical alterations under investigation are neither qualitatively nor quantitatively different in *P. vivax* and *P. falciparum* infections as plotted against (1) the number of days of positive para-

[4] We are indebted to Roche-Organon, Inc. of Nutley, N. J. for generous supplies of Liquaemin (heparin) used in this research.

[5] In order to be able to repeat this determination at more frequent intervals, 3 cc. of the dye was used instead of 5 cc. which has been reported (Feldman and Murphy, 1945) to cause visible "blueing" of the skin and to be a limiting factor in the frequency of this determination. Plasma volumes in some patients were measured every other day for 10–14 days with no serious discolorations appearing.

sitemia, (2) the accumulate number of paroxysms or (3) the number of hours of fever above 103° F. Figures 1 and 2 are presented to illustrate this point. Since the number of days of positive parasitemia is the least equivocal of these variables, all other data have been plotted against it.

Although multiple determinations of the previously listed variables were made on each day of positive parasitemia, we have logically but arbitrarily divided the investigation into five groups of measurements: (1) control-previous to infection with malaria, (2) second to fifth day, (3) sixth to ninth day, (4) tenth to twelfth day and (5) thirteenth to eighteenth day of positive parasitemia.

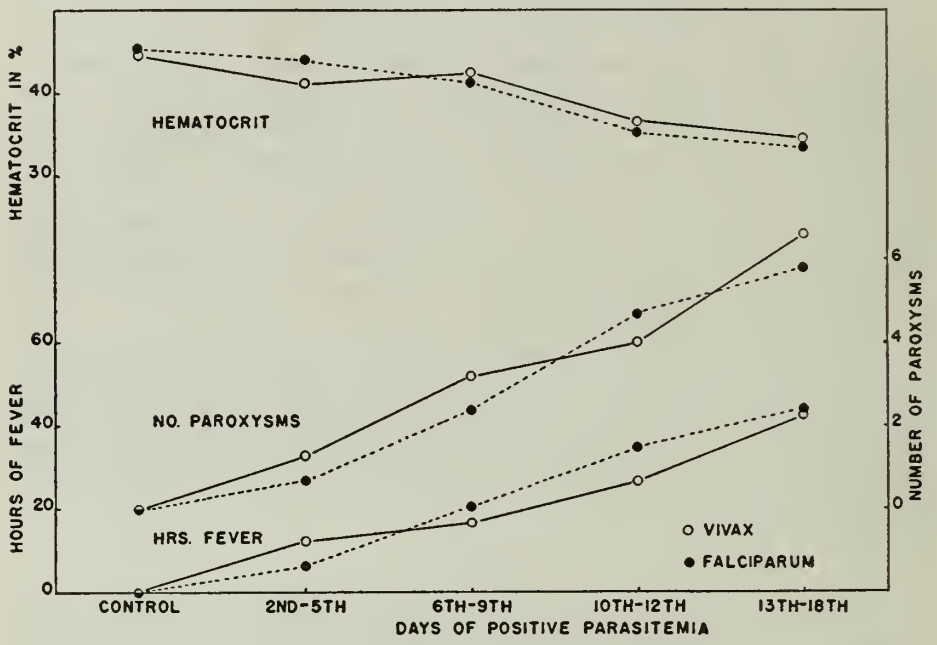


FIG. 1. Comparison of average values obtained in patients therapeutically infected with (1) *P. vivax* and (2) *P. falciparum*.

Since no qualitative and but few quantitative physiological and/or biochemical differences in host reactivity to *P. vivax* and *P. falciparum* infections were found, the results on both diseases have been grouped together. Each point on the graphs presented (except those illustrating individual patients) represents 20–50 separate measurements (see table 1).

The word "control" will be used advisedly throughout the ensuing discussion to designate determinations made on syphilitic patients who had not yet been infected with malaria. Few physiological or biochemical aberrations are seen in the control data from values usually reported as "normal" (table 1). Values for the control blood volume were slightly higher in this group of patients than normal. Whether this is traceable to leutic invasion, to the fact that the negro[6] has a higher blood

[6] Over half the patients used in this study were of the negro race.

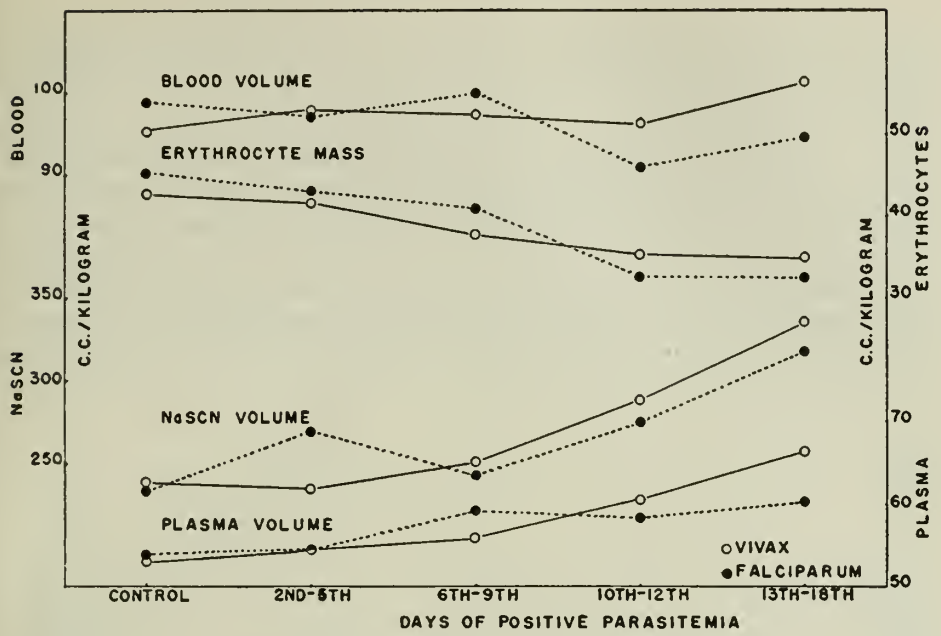


FIG. 2. Comparison of average values obtained in patients therapeutically infected with (1) *P. vivax* and (2) *P. falciparum*.

TABLE 1

*Comparison of average values in groups 1, 2, 3, 4 and 5*

| GROUP NUMBER | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Number of cases | 50 | 29 | 48 | 27 | 19 |
| Days of parasitemia | 0 | 2–5 | 6–9 | 10–12 | 13–18 |
| Parasites per mm.$^3$ | 0 | 6,592 | 15,951 | 21,812 | 11,330 |
| Hours of fever | 0 | 11.4 | 19.7 | 31.6 | 46.4 |
| Number of paroxysms | 0 | 1.1 | 2.8 | 4.3 | 6.8 |
| Plasma Na in m.eq./L. | 149.0 | 150.1 | 146.7 | 151.4 | 149.1 |
| Plasma K in m.eq./L. | 4.8 | 4.8 | 4.6 | 4.6 | 4.7 |
| Plasma Cl in m.eq./L. | 100.0 | 98.6 | 97.2 | 98.8 | 99.4 |
| Red cell Na in m.eq./L. | 15.3 | 15.2 | 15.2 | 15.3 | 16.2 |
| Red cell K in m.eq./L. | 108.4 | 106.9 | 106.1 | 105.6 | 103.8 |
| Red cell Cl in m.eq./L. | 54.4 | 53.1 | 51.5 | 52.3 | 52.8 |
| Urine Na in mM/24 hrs. | 152 | 137 | 88 | 72 | 56 |
| Urine K in mM/24 hrs. | 77 | 80 | 62 | 61 | 44 |
| Urine Cl in mM/24 hrs. | 164 | 142 | 93 | 58 | 55 |
| Plasma volume in cc./Kgm. | 53.7 | 52.5 | 57.4 | 59.8 | 64.8 |
| Blood volume in cc./Kgm. | 97.4 | 94.0 | 98.8 | 93.9 | 99.6 |
| "Extracellular" fluid volume in cc./Kgm. | 236.8 | 240.7 | 253.9 | 289.2 | 330.4 |
| Red cell mass in cc./Kgm. | 43.9 | 41.5 | 40.4 | 34.1 | 34.3 |
| Plasma protein concentration in gms. per 100 cc. | 7.57 | 6.82 | 7.07 | 7.04 | 7.03 |
| Total circulating protein in gms. | 265 | 253 | 255 | 261 | 291 |
| Total circulating protein in gms./Kgm. | 4.12 | 3.86 | 4.00 | 4.16 | 4.43 |
| Hematocrit in per cent | 44.9 | 43.9 | 42.2 | 36.0 | 34.5 |

volume per unit weight, or to the fact that many of the determinations were carried out in summer weather (Conley and Nickerson, 1945) (Overman and Feldman, 1947b) was not ascertained.

Reference to figure 3 will illustrate the method selected in presenting the combined data on *P. vivax* and *P. falciparum* as well as provide a clinical picture of the severity of the disease at each selected point of analysis. The average parasitemia rises almost linearly up to the last group analyzed. The reduced average parasitemia of the patients studied between the 13th and 18th day of positive parasitemia is due to the fact that 12 of the 19 patients in this group had already received anti-malarial therapy and 2 more had a reduced parasitemia following the beginning of spontaneous remission. The average number of hours of fever (over 103° F.) continuously in-

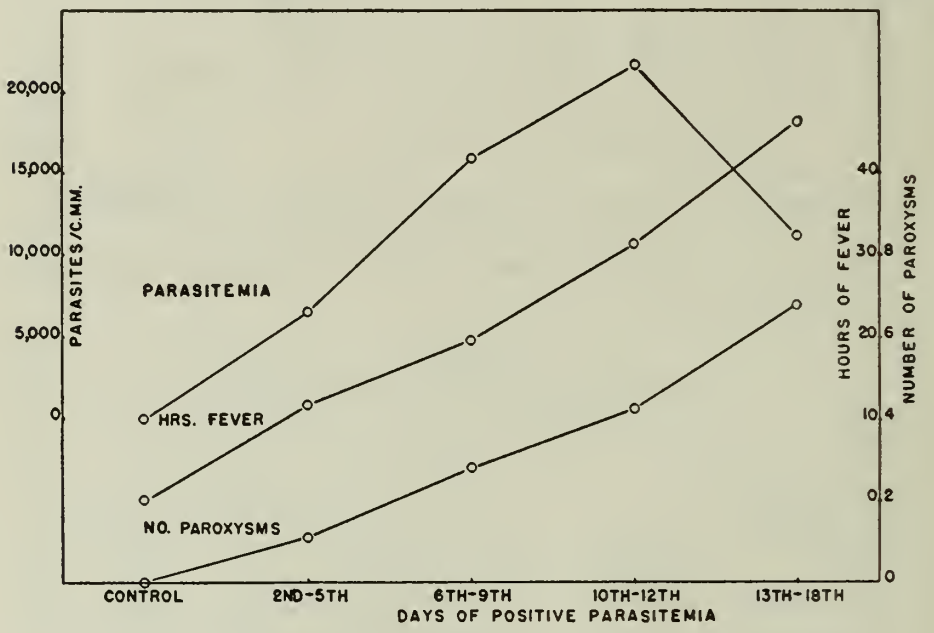


FIG. 3. Combined *P. vivax* and *P. falciparum* data. Average values in 20–50 cases.

creased to 46.4 hours and the average number of paroxysms increased to 6.8 by the 13th to 18th day of positive parasitemia.

*Blood Volume:* As previously reported in simian malaria (Overman and Feldman, 1947) and in a few cases of therapeutic human malaria (Feldman and Murphy, 1945), blood volume alterations in other than terminal or near-terminal malaria are equivocal. Certainly in carefully managed therapeutic malaria no significant or progressive alterations are seen (figure 4). The red blood cell volume reductions which attend the hemolytic crises of malaria are often more than offset by an increase in plasma volume. On the average the dilution of the blood is commensurate with the reduction in erythrocyte mass, the result being an approximate maintainence of the circulating volume. However, as previously mentioned by Feldman and Murphy (1945) and Overman and Feldman (1947), cases in which a profound reduction in

blood volume occurs *were* seen (figure 5). Certain of these patients (e.g. C. C. in figure 5) became sufficiently oligemic to require transfusions of from 500 to 1000 cc. of erythrocytes along with anit-malarial therapy. All such patients made uneventful recoveries. In our series these suddenly fulminating cases are, however, rare and their inclusion in data averages does not materially alter the final results. It may well be that in fulminating human malaria, as in terminal simian malaria, (Overman and Feldman, 1947) blood volume is markedly reduced as postulated by Kniseley, et al. (1945).

*"Extracellular" Fluid Volume:* (Thiocyanate space) The value for the "extracellular" fluid volume in normal human males is given by Crandall and Anderson

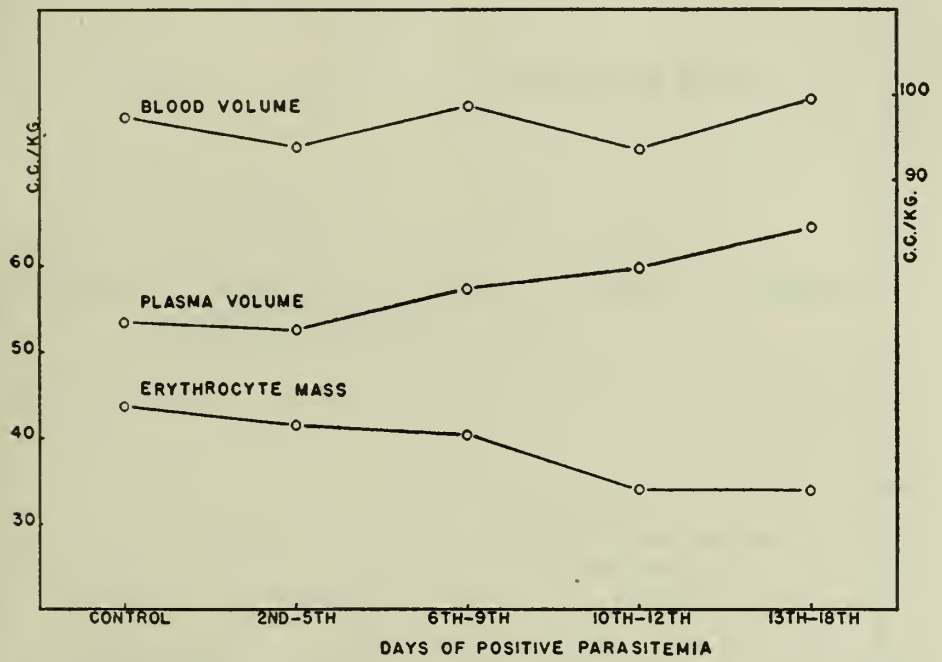


FIG. 4. Average blood, plasma and erythrocyte volume alterations in human malaria (each point represents 20–50 cases).

(1934) as 242 cc. per kilogram or 24.2 per cent of the body weight. Our control value (50 cases) is 237 cc. per kilogram or 23.7 per cent of the body weight. Figure 6 shows that the volume of fluid available for the dilution of SCN increased progressively throughout the course of the disease. This serial increase in SCN volume is qualitatively and, on the average, quantitatively identical to that previously reported in *P. knowlesi* infections in monkeys (Overman, 1946). Actually the grouped data mask some of the more startling alterations of this volume in some patients. Figure 7 illustrates three such cases.

In patient C. D. the "apparent" extracellular fluid volume increased until it was equal to 62.5 per cent of the body weight. Such a figure corresponds closely to the calculated *total* body water (usually given as 65–66 per cent of the body weight).

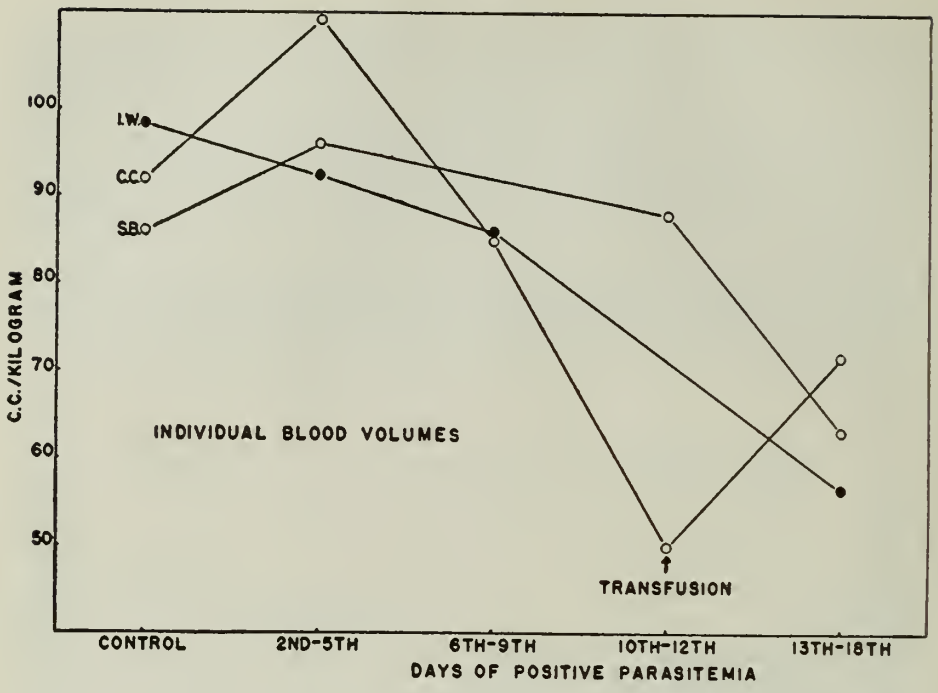


FIG. 5. Individual patient records showing instances of marked blood volume reduction.

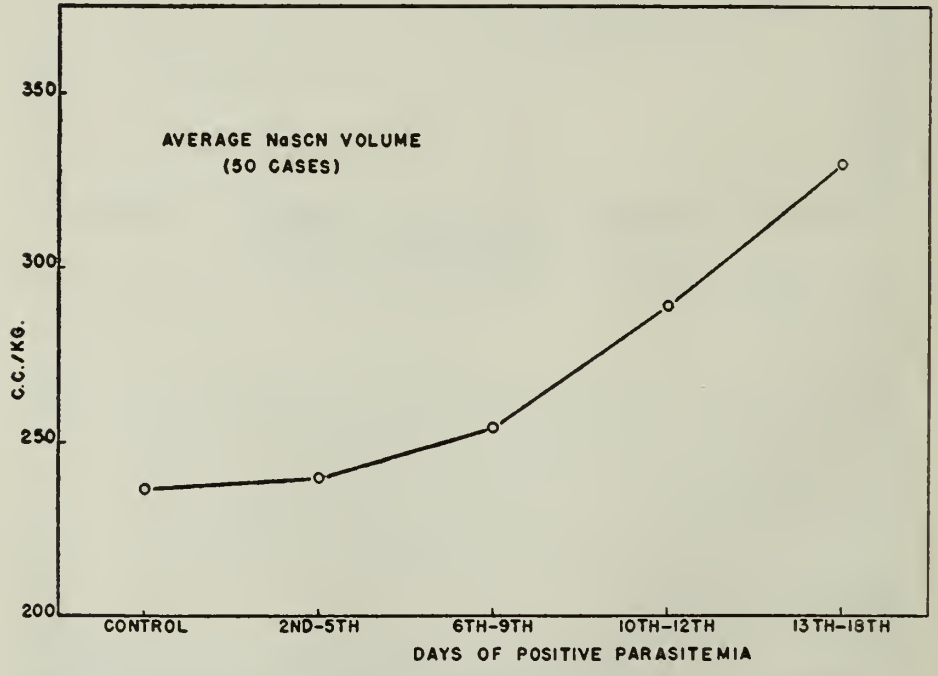


FIG. 6. Showing average alteration in "extracellular" fluid volume in human malaria.

Since there was no increased loss of SCN in the urine and since the patients appeared to be dehydrated, the conclusion is inescapable that in malaria the foreign ion SCN enters the tissue cells and may be eventually diluted by the *total* body water. Similar but more frequent instances have been reported in *P. knowlesi* infections in monkeys (Overman, 1946) (Overman and Feldman, 1947).

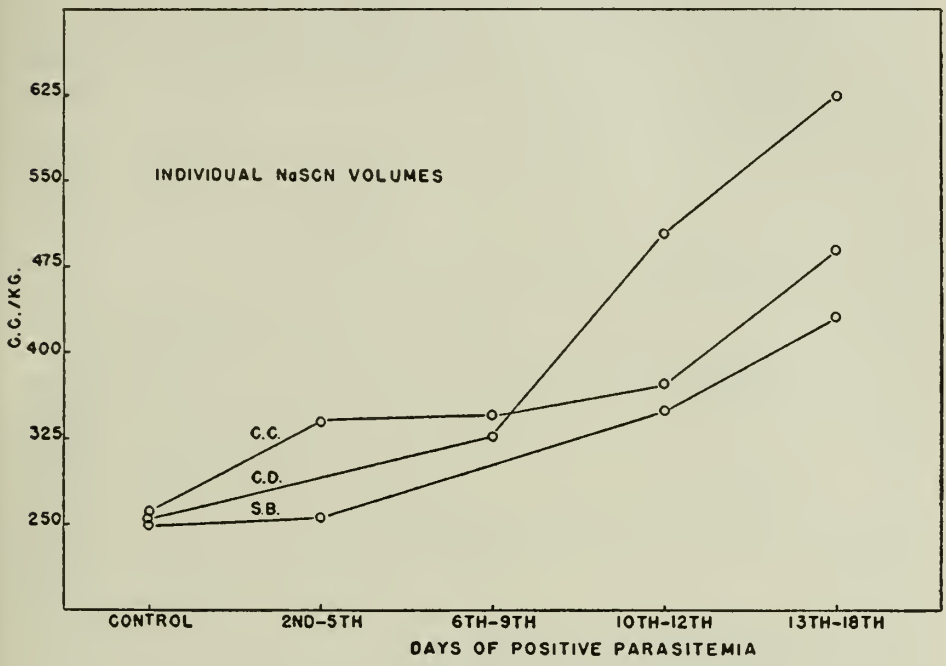


FIG. 7. Individual patient records

Patient C. C., a negro male patient infected with *P. falciparum* showed an 89 per cent increase in SCN volume when he had had 28 hours of fever above 103° F, had sustained 10 paroxysms, had a parasite count of 67,568/c. mm. and a hematocrit of 26 per cent.

Patient S. B., a negro male patient infected with *P. vivax* showed a 73 per cent increase in "apparent" extracellular volume when he had had 56 hours of fever above 103° F and 8 paroxysms, had a parasite count of 12,410/c. mm., and a hematocrit of 41 per cent.

Patient C. D., a negro male patient infected with *P. vivax* showed a 145 per cent rise in SCN volume after the seventh paroxysm when he had had 57 hours of fever over 103° F, had a parasite count of 11,514 per c. mm. and a hematocrit of 29 per cent.

In normal man the SCN ion, like NaCl, is excluded from the cell water. Our data indicates that in malaria the permeability of the cellular membranes is sufficiently altered to permit free passage of the SCN ion, thus introducing a possible new interpretation of previous studies on alterations in *native* ion concentrations in paludic blood.

*Native Ionic Studies:* The indication of altered tissue cell permeability to the foreign ion SCN made desirable a re-investigation and review of native ionic (Na, K and Cl) metabolism throughout the course of therapeutic human malaria. Such serial studies were carried out previously in simian malaria and reported by Overman

(1948). Likewise, numerous scattered observations of alteration in the distribution of Na, K, and Cl in human malaria have been published (Pinelli, 1929; Velick and Scudder, 1940; Zwemer, et al., 1940; Flosi, 1944; Miyahara, 1936; Lahille, 1915; Wakeman and Morrell, 1929; Ross, 1932; Peregrino and Brandão, 1937; Fraga, 1917; Morin, et al., 1934).

The development of flame photometry and the application of this method to Na and K determinations in biological fluids (Overman and Davis, 1947) has greatly facilitated this type of study, and it has now been possible to carry out repeated measurement of these levels in enough cases of therapeutic human malaria to confer validity upon the final results (table 1).

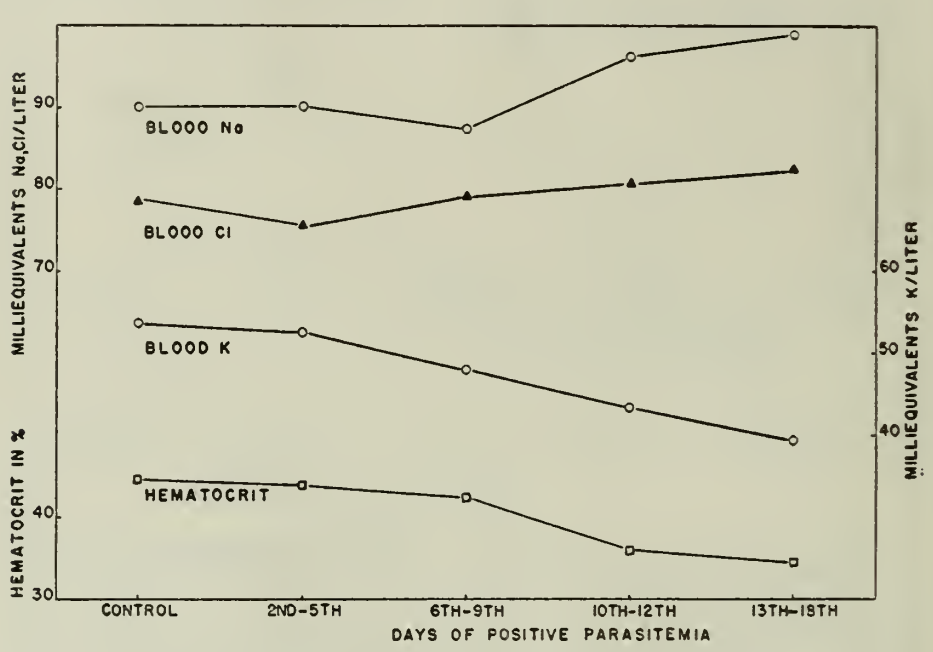


FIG. 8. Average Hematocrit and whole blood ionic changes in human therapeutic malaria

*Whole Blood:* Reference to table 1 and figure 8 confirms the postulate that the levels of Na, K, and Cl in whole blood reflect the progressive anemia. Thus as the hematocrit value declines, the Na level of blood rises and K level decreases. While the average hematocrit fell from 44.9 to 34.5 per cent, the average whole blood Na increased 9.1 meq./L., blood K was reduced 14.2 meq./L., and Cl increased 4.4 meq./L. These average changes are decidedly less marked than those reported for fatal simian malaria (Overman, 1948), although they are qualitatively similar.

*Plasma Ionic Levels:* As in simian malaria, average plasma ionic alterations in therapeutic human malaria generally fail of statistical significance (table 1). Pinelli (1929) reported serum K concentrations in cases of *P. vivax* and *P. falciparum* of 27 to 40.6 mg. per cent (normal, 18 to 20 mg. per cent) during febrile periods and a return to normal values in the apyrexial intervals. Zwemer, Sims, and Coggeshall

(1940) likewise reported a rise in plasma K to 35.2 mg. per cent in therapeutic *P. vivax* infections. Flosi (1944) has similarly reported increases in plasma K and reductions in plasma Na during *P. vivax* rigors. We also have made measurements before, during and following paroxysms in several patients. Each showed a rise in plasma K and a reduction in plasma Na during the paroxysmic phase. However, studies of many cases throughout the malarious course has failed to show any striking *progressive* alteration in plasma ionic levels. If such changes regularly occur during individual rigors, they appear to contribute little permanent alteration to the chemical anatomy of the extracellular fluid. Apparently the kidney remains highly capable of plasma ionic regulation until terminal or near-terminal stages of malaria are reached.

*Erythrocytic Ionic Levels:* From the data presented regarding the distribution of the foreign ion, SCN, in human therapeutic malaria, and from similar studies in simian malaria, (Overman, 1946; Overman and Feldman, 1947) together with the series of studies of native ionic (Na, K, and Cl) concentrations in red cells of the malarious monkey (Overman, 1948), it is reasonable to expect alterations in the cellular distribution of Na and K during the course of *P. vivax* and *P. falciparum* infections in humans. Such ionic changes may logically be expected in the intracellular compartment, which is physiologically protected from renal regulation. Erythrocytes were chosen for analysis as representative cells which can be secured uncontaminated with extracellular fluid, thus obviating the difficulty of interpreting whether the ions measured reside intra or extracellularly.

Because the density of the parasitized erythrocytes is somewhat less than that of the non-parasitized cell, separation of the invaded cells can be obtained by centrifugation. The erythrocytic samples were drawn from the bottom of the centrifuged sample and were chiefly non-parasitized cells.

Reference to figure 9 will show a linear reduction in the average non-parasitized erythrocyte K concentration over the course of the disease and an almost equal increase in cell Na. Both of these average changes fail of statistical significance, but each is qualitatively identical to the highly significant changes in ionic anatomy of the non-parasitized erythrocytes of the fatally infected monkey (Overman, 1948). Failure of the ionic changes in these human cases to reach the point of high statistical significance lies, we believe, in the necessarily minimal and carefully controlled nature of therapeutic malaria.

Again, however, as with the data on SCN distribution, the presentation of averages tends to mask certain striking cases which so closely parallel the changes previously reported in fatal *P. knowlesi* as to warrant separate presentation. Figure 10 illustrates the erythrocyte Na alteration which took place in four individual cases.

Similar data can be presented for erythrocyte K. Although the graph (figure 9) illustrates a relatively insignificant *average* reduction in cell K, individual patients in whom large increments in cell Na were found showed almost equal (in meq./L.) cell K reductions. Thus individual cases, as well as average data on 50 to 60 patients, bear out the general contention previously stated with regard to *P. knowlesi* infections in monkeys, i.e., that it would appear that there is an exchange of Na for K in uninvaded erythrocytes in these diseases.

Although no attempt will be made at this time to present complete data on the

convalescent course of these patients (such studies have been made and are being prepared for publication), it is well to point out that, just as the native ionic alterations are reversible (see figure 10), so are the changes in the distribution of the foreign ion, SCN (figure 11). The change in cellular permeability to ions, foreign or native, is not, however, *immediately* reversible but requires weeks or months for readjustment—weeks or months which roughly coincide with the period of subjective complaints of post-malarial debility. The *P. knowlesi* infected monkey, which presents a far more fulminating disease, requires an even longer period for cellular convalescence (Overman, 1946).

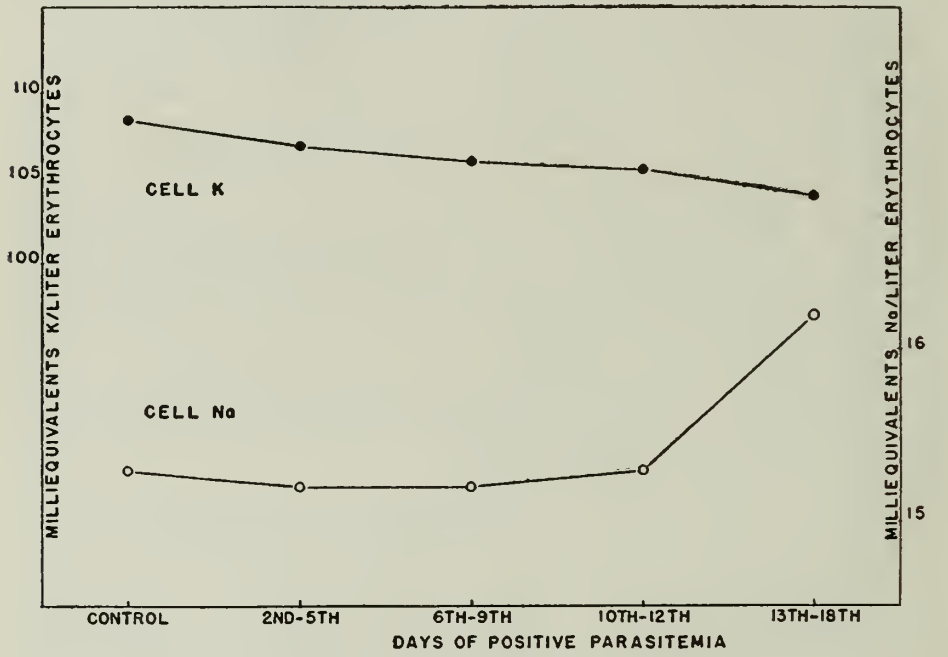


Fig. 9. Average Na and K concentration changes in erythrocytes

*Urinary Excretion of Ions:* If Na and Cl enter the cells of the malarial host, the fact that the plasma levels of these ions remains constant over the course of the disease leads one to expect profound alterations in the urinary excretion of these elements. It has been previously reported by Overman (1948) that such severe changes do occur in fatal simian malaria. In a specific animal, for instance, the total urinary Na and Cl output fell from normal values of 480 and 640 mM per 24 hours respectively to 10 and 40 mM by the time the animal bore an 84 per cent parasitemia. Concomitantly, urinary K rose from 420 to 740 mM per 24 hours and only fell again as the animal became severely oliguric.

Some of these reported alterations in fatal simian malaria are qualitatively paralleled by the human host with therapeutic *P. vivax* and *P. falciparum* infections. Reference to figure 12 will show that average reductions in total Na and Cl output over the malarious course were 63 and 66 per cent respectively. These changes are

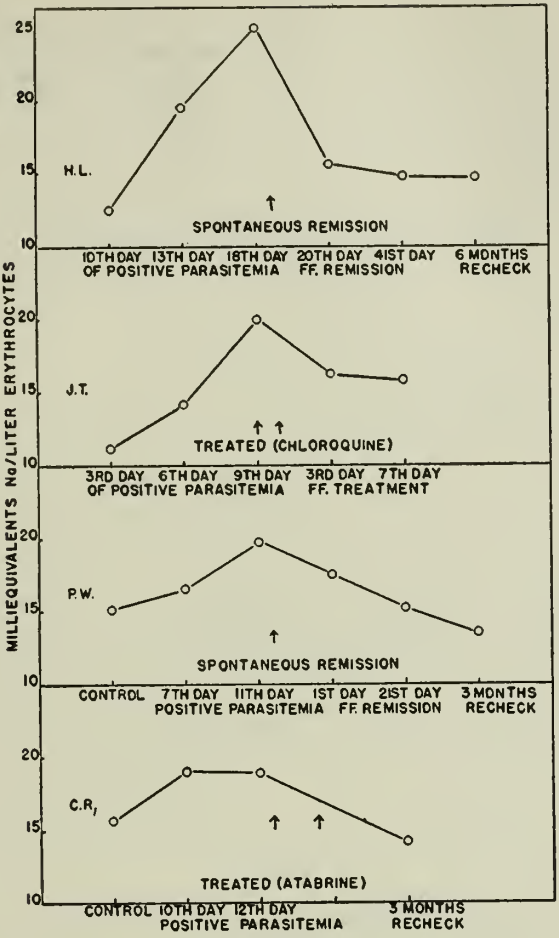


FIG. 10. Alterations in erythrocytic Na concentration in individual patients

Patient H. L., infected with *P. vivax*, showed a 100 per cent increase in erythrocyte Na between the 10th and 18th days of positive parasitemia. This patient had a spontaneous remission, following which he was studied intermittently for 6 months. At the time of the 6 month recheck, the cell Na had not yet returned to control level.

Patient J. T., infected with *P. falciparum*, showed a 73 per cent increase in red cell Na by the 9th day of positive parasitemia. He was treated with chloroquine and had no fever by the third day following therapeutic intervention. However, the cell Na level was still 37 per cent above normal on the 7th day following the beginning of treatment.

Patient P. W., infected with *P. vivax*, showed an increase in red cell Na of 33 per cent on the 11th parasitemic day. This patient had a spontaneous remission and had returned to normal cell ion balance by the 21st day following remission.

Patient C. R., infected with *P. falciparum*, displayed only a moderate (23 per cent) rise in cell Na, was treated with atabrine between the 12th and 14th days, and had returned to a level 1.5 meq./L. below control value three months later.

by no means so severe as those reported in the monkey, but again the disease process in these patients was relatively mild. Unlike the data reported in the simian disease,

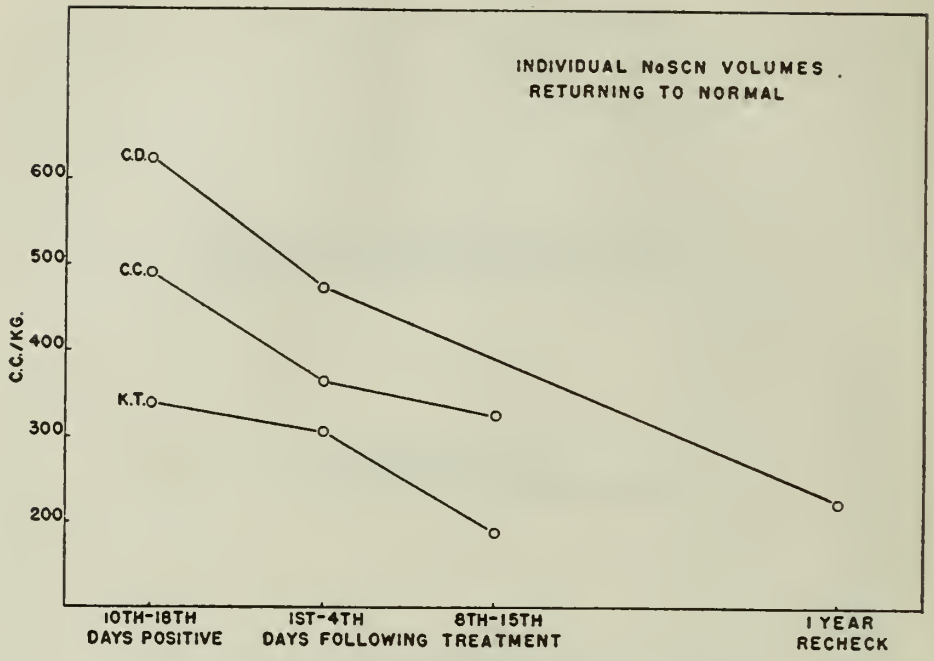


FIG. 11. Individual patient records during cellular convalescence

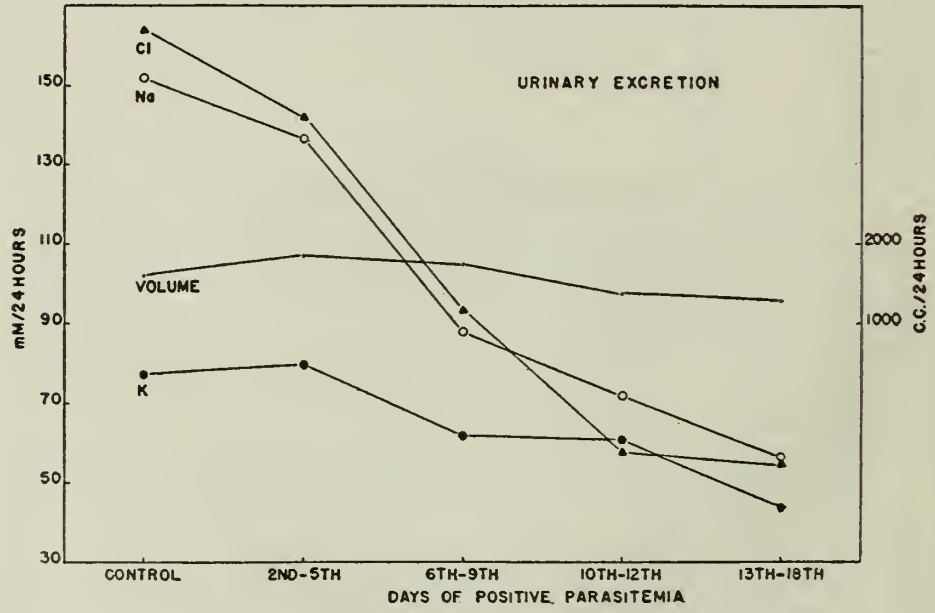


FIG. 12. Alterations in urinary volume and the excretion of ions

the 24 hour urinary K excretion in this series of human subjects also fell throughout the malarious course to reach a figure representing a 43 per cent reduction from

control values. The possibility remains that *average* increases in K excretion might be seen in more severe human disease. Individual cases were followed in which large increments in K excretion occurred. At least one such patient showed a progressive increase in total urinary K which reached a value 8.5 times the control level.

*Plasma Proteins:* There have been numerous previous observations of the hypoproteinemia in human malaria (Boyd and Proske, 1941; Kopp and Solomon, 1941; Feldman and Murphy, 1945). No attempt was made in the present study to assay levels of individual protein components, but the expected reduction in concentration of total protein was found, as shown in figure 13.

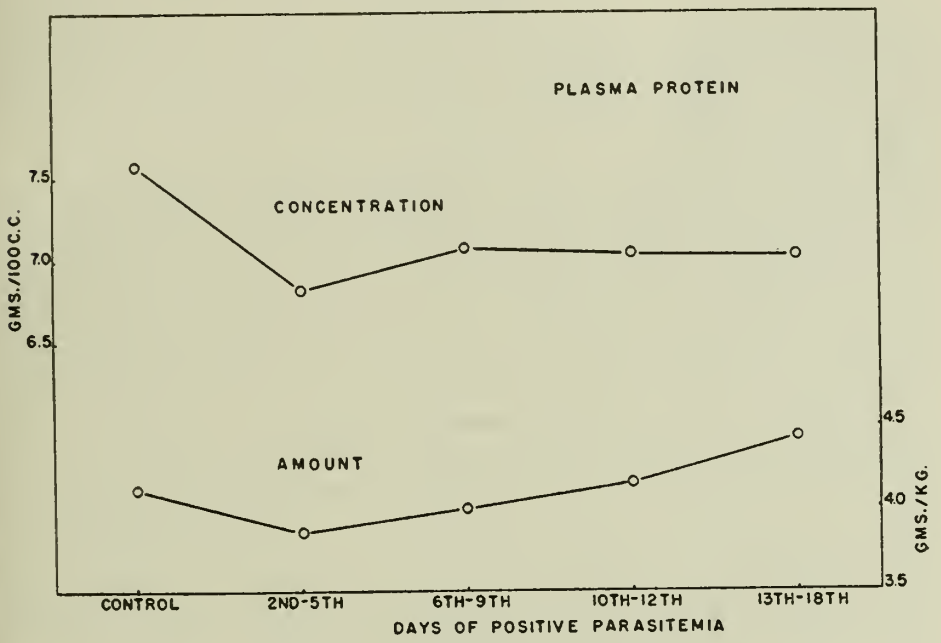


FIG. 13. Average changes in plasma protein

Since the ability of a patient or an animal to dilute his own blood with interstitial fluid in instances of hypotension, as frequently occur at the onset of the sweating stage of the malaria paroxysm (Emerson and Dole, 1944), depends not only upon the concentration of plasma protein but also upon the *amount* of circulating protein, data is presented on total circulating plasma protein per kilogram body weight (figure 13).

On the average, the patients studied present no picture of consistent weight loss (table 1), and thus the rise in average circulating protein per kilogram must be a reflection of the increased plasma volume. It is evident that both water and protein are added to the blood over the malarious course and that the reduction in protein concentration is caused by the addition of somewhat more water than protein in the dilution process. This point is important since the hypoproteinemia of malaria has often been interpreted as presaging increased capillary permeability to blood protein. The loss of albumin, which undoubtedly occurs in malaria, has likewise been so interpreted. However, recent studies which show that albumin is normally filtered

in the kidney and then reabsorbed tend to discredit even this assumption since renal tubular impairment is a well-known malarial sequel. Certainly if the total amount of circulating plasma protein increases, no physiological importance can be attached to possible local leakage of protein from the vascular system.

We are in agreement with Feldman and Murphy (1945) that the anemia of human malaria has a two-fold origin: (1) actual parasitic destruction of erythrocytes and (2) dilution of the blood, which almost certainly is an intermittent process coincident with the paroxysmic periods of hypotension.

## DISCUSSION

Many of the problems arising in the interpretation of these data have been discussed previously relative to similar measurements made in groups of monkeys infected with *P. knowlesi* (Overman, 1946; Overman and Feldman, 1947; Overman, 1947; Overman, 1948).

The general physiological picture exhibited by both the simian and the human malarial hosts is as follows: erythrocytic destruction, which uncompensated would rapidly lead to oligemia and circulatory failure, provokes dilution of the blood with tissue fluids, possibly through intermittent bouts of hypotension as the vasomotor centers are affected during paroxysms. Protein, as well, is added in the dilution process. Toxic products of parasite metabolism and/or the effects of parasite metabolism on host response (such as depression of the activity of the adrenal cortex) bring about alterations in cellular membrane permeability such that cell walls, previously impervious to Na and Cl entrance, become progressively permeable. The resulting tendency for reduction in extracellular concentrations of these ions is largely offset (at least in cases of mild disease) by the renal tubular reabsorption of a great fraction of the filtered Na and Cl. Particularly in man, this observed renal action may be a response to salt loss by sweating as well as Na and Cl "loss" by cell entrance. On the other hand, the monkey, which possesses a far less efficient sweating mechanism, displays the same renal activity to an even more marked extent. It cannot, therefore, be postulated that sweating alone is the stimulus for the observed marked reduction in renal excretion of Na and Cl.

The absolute importance of cellular permeability alterations in malaria is, as yet, unknown. One may postulate, however, that changes in intracellular ionic anatomy to the extent described can scarcely fail to produce drastic alteration in the operation of cellular enzyme systems, which normally are the basis for all cellular activity. Whether the alterations which we have measured are a simple deleterious reflection of parasitic metabolism or whether they constitute an organic chemical "reflex" of a protective character is, as well, unknown. It might be remarked that in general the species of animals which are naturally or can be experimentally infected with malaria are those in which the erythrocyte Na level is normally low (i.e., man, monkey, bird) whereas the carnivores, normally possessing a relatively high red cell Na concentration, are uninfectable with malaria.

Mention has been made of possible involvement of the adrenal cortex. This is, of course, not a new idea although most previous suggestions of adrenal cortical involvement have been based solely or largely on grounds of clinical observations. Recently

we have been able to study serially 2 groups of monkeys indentically infected with *P. knowlesi*, one of which was treated daily with whole adrenal cortical extract. The resultant prolongation of life and amelioration of observed biochemical changes in this group will be discussed elsewhere (Overman, 1949).

It is not our belief that alterations in cellular permeability to Na, Cl, and K *alone* constitute an all-important factor leading to death. In a syndrome as complex as malaria, the total physiological response of the host must be considered. Lack of progress in the treatment not only of fulminating epidemic malaria but of chronic relapsing disease as well may quite possibly lie in our general proclivity to look for and attempt to alter but one or at most a few host variables. The study of terminal processes in malaria, while valuable if properly assayed, has often led us away from a catholic view of host response into an erroneous tendency to attribute fatal outcome to anemia or peripheral anoxia, or some other single phenomenon.

Finally it is perhaps well to point out again that many biochemical and physiological alterations in the host, which might be considered deleterious do not necessarily coincide in time with the more usually observed and measured clinical signs of disease. In general, most of the host responses presented here outlast the usually accepted clinical signs. Reference to figure 3 will show, for example, that the group of observations made on the 13th to 18th day of positive parasitemia (which are, generally speaking, the most aberrant values shown) were made at a time when the average parasitemia had already been reversed either by chemotherapeutic intervention or following spontaneous remission. Likewise, reference to the individual graphs of erythrocyte Na alteration (figure 10) or those concerning the return of SCN volume toward normal (figure 11) will reveal that such aberrations are still manifest weeks or even months following what might be termed clinical convalescence by the absence of parasites, fever, chills, etc.

It would seem possible, therefore, that at least in malaria, it would be well to differentiate between "clinical convalescence" on the one hand and "cellular convalescence" on the other, just as we already differentiate between "clinical death" and "cellular death."

## SUMMARY

1. More than 250 determinations each of the following variables were made in 70 human patients therapeutically infected with *P. vivax* (McCoy) and *P. falciparum* (Costa): plasma volume (total and per kilogram), blood volume (total and per kilogram), erythrocyte mass (total and per kilogram), "extracellular" fluid volume (total and per kilogram), plasma Na, K, and Cl, whole blood Na, K, and Cl, erythrocytic (non-parasitized) Na, K, and Cl, parasitemia, urinary concentration of Na, K, and Cl, 24-hour urine volume, plasma protein concentration, total circulating plasma protein, hematocrit, and body weight.

2. Data on alterations in these host variables during the active phases of the diseases are graphically presented and discussed.

3. The relations between human host response to therapeutic *P. vivax* and *P. falciparum* and the simian host response to fatal *P. knowlesi* are delineated.

4. Evidence of altered cellular permeability to Na, K and Cl is presented and the

reversible nature of this alteration reviewed in the light of concomitant disturbances in other physiological phenomena.

ACKNOWLEDGEMENTS

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The facilities of the Gailor Psychiatric Hospital, Memphis, were used in housing and caring for the patients studied.

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# A SUMMARY OF THE EXPERIMENTAL USE OF DDT AS A MOSQUITO LARVICIDE

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## INTRODUCTION

A summation and digestion of the literature on the experimental use of DDT as a mosquito larvicide is opportune, since the developmental stage of this larvicide has now merged with the utilitarian or operational phase. Agencies such as the U. S. Public Health Service and the Tennessee Valley Authority now advocate the widespread use of DDT larvicides. Numerous mosquito control districts and municipalities likewise now incorporate the new technique in their sanitation programs. Other compounds, some of which are related to DDT are now being studied on an experimental basis, and have been found equal to, or more potent than DDT. Some of these may replace DDT in its present widespread use as a mosquito larvicide. Experimentation with such compounds may be expected to follow the basic design developed for DDT larviciding studies with the probable maintenance of DDT potency as a standard of testing for some time to come.

DDT mosquito larviciding is a valuable adjunct to the more striking residual wall treatment directed against adult mosquitoes. Larviciding should continue to play an important role either in the control or the eradication (Lyle, 1947) of pest or disease vector species of mosquitoes.

## HISTORY

Possibly the first reference in this nation's literature to mosquito larviciding appeared as an editorial, August 29, 1793 (Anon.), in a Philadelphia newspaper. To Dr. L. O. Howard (1892) belongs the distinction of having instigated experimental and educational programs with respect to the oil-film method of killing mosquito larvae. The first use of DDT as a mosquito larvicide was orally reported to the authors by August Wiesmann[1] who remarked upon the striking potency of the material as used against Swiss culicines previous to American reception of the material. Concerning original studies, Dr. Victor Froelicher[2] has asserted in a personal communication (1947): "No early work was published on the use of DDT as a larvicide (mosquito) in Europe, to our knowledge." The general experimental use of DDT dates from November 1942, when samples of *Gesarol Spray Insecticide* (DDT 5 per cent) and *Gesarol Dust Insecticide* (DDT 3 per cent) were sent to the U. S. Department of Agriculture workers from the European agent of the Geigy Co.,

[1] Swiss agricultural entomologist who discovered the insecticidal properties of DDT.

[2] Director of Research, Geigy Company.

J. R. Geigy, S. A. Basle, Switzerland (Anon., 1944 (c); Knipling, 1945). On the basis of the realization of the value of the compound as a general insecticide, larviciding studies, as well as others, were instituted in February 1943, at the Orlando, Florida laboratory of the Bureau of Entomology and Plant Quarantine under Deonier and his co-workers (1945(a); 1945(c); 1946(a); Eide, et al., 1945(a)). A supply of DDT had been allocated by the War Production Board for research during this period (Essig, 1945 (a)). In the process of these preliminary studies a patent pertaining to DDT larviciding and dedicated to U. S. Government ownership was issued (Deonier, et al., 1944). Essig (1945(a)) notes that the first six months of 1943 were a period of intensive study on methods of mosquito killing by the Orlando, Florida group. The results of these preliminary tests first appeared in print in the Journal of Economic Entomology (February 1944). An oral report was made by Stage (1943) on these early field larviciding tests. Jones and his co-workers (1945) had previously prepared a report on usable DDT larviciding preparations. It was not until March 1945 that the general public heard radio presentations on the subject of DDT (Winternitz, 1945). Herms and Gray (1944) in early preliminary laboratory tests observed the residual characteristic of DDT on experimental glassware. These findings were paralleled by those of Wasicky and Unti (1944) during the same period. At about the same time, Lindquist and Bushland (1944) reported upon the high degree of effectiveness of DDT larvicides used against *Chaoborus* (a mosquito relative) in field studies. Preliminary observations on the possible effects of DDT on wildlife appeared in *Science* (Ellis, 1944). Possibly the first reference to the extensive use by the Armed Forces of the new mosquito larvicide is that of Farr (1945). This relates to the efforts of the Fifteenth Air Force in Italy to fit DDT into their mosquito control program. The first large scale operational use of DDT oil-mist larviciding occurred under the direction of J. D. Taylor[3] (Anon., 1946(a)) as part of the State of Arkansas' fight against malaria. This initial control program in 1946 was, in part, based on the analysis of an extensive experimental study (Mathis, et al., 1947) by the U. S. Public Health Service made during the previous mosquito breeding season in Georgia. The substitution of DDT oil-mists for oil sprays occurred in the national malaria mosquito control program of the U. S. Public Health Service in April 1946 (Henderson, et al., 1946).

(*References: 1, 4, 6, 7, 10, 30, 32, 34,* (DDD), *37* (BHC), *38, 41, 43, 47, 61, 63, 66, 72, 77, 84, 88, 89, 101, 102, 103, 104, 105, 121, 128, 131, 140.*)

## PRELIMINARY PAPERS

Certain papers in the literature on this subject are designated as preliminary. Such reports have served well their function of education and stimulation of additional studies. Many of them are marked by the promissory nature of their contents in relation to future use of the new compound. Some of them were obviously designed for lay consumption.

(*References: 2, 3, 4, 5, 8, 14, 19, 20, 44, 52, 55, 63, 77, 90, 92, 109, 110, 111, 128, 129.*)

[3] Sanitary Engineer, Arkansas State Board of Health.

### GENERALIZED PAPERS

Another group of papers has been classified as generalized since they have been written for reception by groups such as pest control personnel, agricultural workers, etc, whose interest in larviciding is incidental. Some have described the use of DDT in the Army's mosquito larviciding program. A few are completely generalized for inclusion in various encyclopedias. A fair number of reports on DDT larviciding have been made by laboratory directors at national meetings of various health organizations. Such generalized reports represent a more stable, usable source of information than those cited above as preliminary.

(*References: 13, 18, 21, 24, 41, 42, 60, 76, 111, 115, 117, 119, 131, 136.*)

### DDT LARVICIDE DOSAGE RECOMMENDATIONS

Due to the extreme potency of DDT as a mosquito larvicide, the problem of the proper treatment dosage has received profound consideration. In all of the larviciding agencies involved in such research a careful effort has been made to arrive at a DDT dosage per treatment which would be reasonably compatible with the goals of wildife conservation and at the same time produce satisfactory larval kills. The problem is a highly complex one in which a multitude of factors enter. The U. S. Department of Agriculture has recommended a dosage of no more than 0.1 pound of DDT per acre (Eide, et al., 1945(a); Knipling, 1945 (b); 1946; Lindquist, et al., 1945(a)). The U. S. Public Health Service has felt that not more than 0.05 pound of DDT per acre should be routinely used in hand larviciding where aquatic wildlife is of importance (Ferguson, et al., 1947; Mathis, et al., 1947; Simmons, et al., 1945(d)). A dosage of 0.1 pound per acre has been found safe for plane larviciding. The U. S. Fish and Wildlife Service has indicated (Cottam, et al., 1946(c)) that less than 0.5 pound of DDT per acre should be used to avoid harming fish and fish food organisms; less than 2.0 pounds per acre should be used to avoid damage to birds, amphibians and mammals in forested regions. The Tennessee Valley Authority group has shown that dosages of 0.1 pound of DDT per acre give no evidence of harm to fish or aquatic organisms when applied by plane (Metcalf, et al., 1945). The problem of the effects of repeated doses has been handled by Tarzwell (1947).

(*References: 29, 38, 45, 78, 79, 85, 93, 118.*)

### HEALTH HAZARDS WITH DDT LARVICIDING

DDT dissolved in oils and certain other organic solvents is toxic by means of skin absorption. Henderson and Bradley (1946) have outlined the reservations necessary for the safe handling of DDT larvicides. Such practices are illustrated in the DDT larviciding training film of the U. S. Public Health Service (Anon., 1946 (b)).

In general, it appears that with a reasonable degree of precaution, DDT larvicides may be used without danger to one's health. Stilson (1946) observes that in tests concerning the military use of DDT larvicides in the Okinawa invasion, an officer drank one quart of water daily for three weeks containing 0.5 ppm DDT with no apparent ill effects. On the other hand, there has been reported a death from drinking

120 cc. of 5 per cent DDT in kerosene (Smith, 1948). Its possible retention in the soil in concentrated dosages has recently been considered (Wilson, et al., 1946).

(*References: 5, 9, 13, 61, 120, 123, 130.*)

## SOURCES OF INFORMATION ON DDT LARVICIDING

The chief sources of information in this subject are to be found in the following publications: Public Health Reports and its Supplements, Bulletins of the U. S. Public Health Service, Journal of Economic Entomology, Mosquito News, the valuable Roark bibliographies and digests (Roark, 1944(a); 1945(a); 1945(b); Roark, et al., 1944(b); 1946), various public health training films and manuals[4] (Anon., 1946(b); 1946(c); 1945(b); 1945(c)) and the Abstract Bulletins (Nos. 1–12, December 1945–January 1947) of the National Research Council Insect Control Committee[5]. Other sources, less complete, are: Proceedings of the California Mosquito Control Association, Journal of the National Malaria Society, Bulletin of Arkansas Malaria Control Program, American Journal of Tropical Medicine, American Journal of Public Health, Soap and Sanitary Chemistry, Science, Biological Abstracts and Chemical Abstracts.

## POTENCY OF DDT LARVICIDES

At the time of their introduction DDT larvicides were the most potent known (McCoy, 1944(b)). This has been shown by a multitude of field and laboratory tests in recent years. Bishopp (1946(a)) has remarked upon this factor: "Prior to the discovery of DDT, phenothiazine was the most effective substance employed against *Culex* mosquito larvae and DDT appears to be almost 100 times as toxic as phenothiazine to larvae of *Anopheles quadrimaculatus* Say." DDT has been tested in comparison with various formerly used materials and in all cases has proved to be equal to or more potent than any one of them. It has been tested against phenothiazine (Bishopp, 1946(a); Deonier, 1945(b); Eide, et al., 1945(b)), pyrethrins (Lindquist, et al., 1944), and various oils used alone (Deonier, et al., 1945(a); Dews, et al., 1946; Ginsburg, 1945(c); Henderson, et al., 1946; Lever, 1944; Metcalf, et al., 1945, Wisecup, et al., 1945(b)). It is certain that emulsions (Deonier, et al., 1945(c); Ferguson, et al., 1947; Ginsburg, 1945(c); Mathis, et al., 1947) and certain suspensions (Arnold, et al., 1945; Eide, et al., 1945(b); Ferguson, et al., 1947) are decidedly more toxic in comparison to other types of DDT dispersion. Wisecup (1945(b)) notes that in general 0.1 pound of DDT per acre is at least as effective as 1.0 pound of paris green per acre. Mathis, Ferguson, and Simmons (1947) have shown that a 1 per cent DDT dust, compared to a 10 per cent paris green dust (applying each at a rate of 10 pounds of finished dust per acre), gives approximately the same degree of larval control on large anopheline larvae (third and fourth instars) and a slightly higher rate of reduc-

[4] A recent manual (DDT and other insecticides and repellents developed for the Armed Forces. U. S. D. A. Misc. Publ. 606, Aug. 1946) contains a useful summary on general larviciding in Part III with special emphasis on airplane DDT dispersion equipment.

[5] No reference is made in this paper to the many valuable summaries contained in this unpublished series.

tion on small larvae (first and second instars). Moreover, either the DDT-oil-water emulsion or the DDT-oil-mist spray gives a higher degree of control of all instars considered separately than does either the paris green or DDT dust larvicide. Some information is available on the relative toxicity of the DDT isomers (Jones, et al., 1946(c)). Preliminary reports indicate that certain compounds have an equal potency (Benzene Hexachloride) or even greater potency (DDD or TDE) than DDT (Deonier, et al., 1946(a); Dumbleton, 1945).

(*References: 5, 10, 15, 17, 30, 31, 32, 33, 34, 35, 37, 39, 44, 45, 51, 61, 62, 73, 75, 78, 81, 83, 84, 93, 96, 97, 118, 121, 128, 133, 137.*)

### DDT POTENCY AS A PUPICIDE OR OVICIDE

Mosquito pupae exhibit a remarkable resistance to DDT larvicides; the reason for this is as yet unknown. Culicine pupae are resistant to the ordinarily potent DDT emulsion even when applied at from 2.0 to 4.0 pounds of DDT per acre (Ginsburg, 1945(c)). However, it appears that pupal kills are greater with oil emulsions than with dusts or sprays (Ginsburg, 1945(a)). Arnold, Ferguson and Upholt (1945) working with several types of DDT larvicides have remarked upon the apparent lack of pupal mortality in their field tests. In early laboratory studies, Wasicky and Unti (1944) noticed in experiments involving a dosage of 1 part DDT to 60 million parts of water, that this did not kill the anopheline eggs nor prevent egg laying, but all larvae died immediately upon hatching. Upholt, et al. (1945), working with *Aedes aegypti* has observed: "Pupae were exposed to concentrations of DDT as high as 4 ppm as a xylene emulsion. At these higher dosages a high mortality of pupae occurred in the solvent check, indicating that the xylene itself was sufficiently concentrated to kill pupae. At all lower dosages there was a certain mortality of pupae but in no case was it markedly greater than the mortality in untreated checks. There seemed to be a marked tendency for the mosquitoes to die in ecdysis either while pupating or when emerging as adults." Lindquist and Bushland (1944) have shown that while dosages of 1 part in 75 million parts of water are lethal to *Chaoborus* larvae (a mosquito relative) this must be doubled to kill pupae.

The subject of the relative resistance of the various larval instars has interested those working in large scale field studies. DDT oil-mist sprays have been shown to be equally effective against all larval instars (Ferguson, et al., 1947) while DDT dusts seem to be slightly more effective in killing the smaller instars than it does the larger ones (Mathis, et al., 1947). Eide, et al. (1945(b)), has noted some difference in the reactions of first instars as compared to fourth instars, the first instars being more susceptible to certain DDT formulations.

(*References: 15, 31, 49, 51, 84, 126, 128.*)

### DDT LARVICIDE TOXICITY VS. DISTRIBUTION OF PARTICLES

One of the basic factors concerned in DDT mosquito larviciding is that its particulate distribution and subsequent toxicity is limited to the distribution of the solvent or diluent used. Arnold and his co-workers have furnished data concerning this (Arnold, et al., 1945). The idea that pouring of DDT solutions on one part of a

watered area will affect larvae in untreated areas is unsound, unless conditions are such that the solution will spread to all parts of the area.

### DDT LARVICIDING RESIDUUM

The attainment of an effective residual larviciding treatment compatible with aquatic wildlife conservation in natural waters is as yet a goal. Several references have been made to the development of residual treatments in quiet, isolated mosquito breeding pools which receive high concentrations of DDT (Knipling, 1945(b); 1946; Lackey, et al., 1945; Ribbands, 1945; Upholt, et al., 1945). There are scanty references (Deonier, et al., 1945(a); Dews, et al., 1946; Knipling, 1946; Wisecup, et al., 1946) on the use of extreme dosages (from 0.3 pound to 2.0 pounds per acre) in civilian larviciding tests. A remarkable residuum develops upon contact of DDT solutions with laboratory aquaria or glassware (Herms, et al., 1944; Lackey, et al., 1945; Upholt, et al., 1945). Upholt (1947) has advanced a theory as to a possible reason for a lack of field larviciding residuum. The theory is essentially based on the absorption of DDT upon the organic soil components of a pond bottom complex. Sandy soils with a minimum of organic material are the poorest DDT absorbents. The redistribution of DDT due to wind and wave action with the ultimate precipitation of particles is also a significant factor.

(*References: 8, 9, 17, 30, 36, 38, 39, 60, 63, 70, 78, 79, 80, 82, 85, 98, 115, 119, 124, 126, 127, 128, 132, 135.*)

### RELATIVE DDT LARVICIDING EFFECTIVENESS WITH VARIOUS MOSQUITO SPECIES

The literature in general indicates no significant difference in the reactions to DDT larviciding among the several types of mosquito larvae. Ginsburg (1945(c)) notes poor control on sub-surface feeding culicines treated with dust mixtures. In foul, dirty water, Hurlbut and Bohart (1945) have shown culicines to be more difficult to kill than in clean areas. Knipling (1946) thinks it necessary to use dosages higher than five quarts of a 1 per cent solution per acre to kill culicines in comparison to the readily killed anopheline larvae. Ribbands (1945) shows in his work that a longer residual action develops with anopheline than it does with culicine forms. Working with *Culex annulirostris* and *Anopheles farauti*, Travis (1946) did not observe any difference in their responses to DDT larviciding.

(*References: 32, 39, 49, 51, 63, 65, 67, 70, 79, 80, 83, 84, 85, 98, 121, 125, 126, 134, 135, 137.*)

### DDT LARVICIDING IN RELATION TO WILDLIFE

No phase of the general insecticidal use of DDT has received more careful consideration than the possible harmful effects of larviciding upon animal forms native to mosquito breeding areas. The physical state in which DDT is dispersed is very important in this respect. Dusts are relatively harmless (Eide, et al., 1945(a); Horsfall, 1946; Metcalf, et al., 1945), but emulsions (Deonier, et al., 1945(a); Ginsburg, 1945(b); Tarzwell, 1947) are extremely toxic to aquatic organisms. Oils are especially harmful to surface forms (Eide, et al., 1945(a); Henderson, et al., 1946; Met-

calf, et al., 1945). A most conservative view on the subject has been expressed by Cameron (1945): "At a concentration of as low as 1:10 million, it (DDT) can kill fish and many aquatic invertebrates, and while this is higher than is necessary to kill mosquito larvae, the factor of safety is not very great. The armed forces (Canadian) regard this danger as considerable, specifying that DDT will not be used for this purpose except for specifically designated projects." Cottam (1946(c)), expressing the views of the U. S. Fish and Wildlife Service, takes a milder attitude advising the use of less than 0.5 pound of DDT per acre "to avoid damage to fish, crabs, or crayfish; less than two pounds an acre to avoid damage to birds, amphibians and mammals in forest areas. Do not use emulsions." In general it is now felt that DDT may be effectively used with lower dosages (0.05–0.1 pound per acre) with a minimum harm to other organisms. Tarzwell (1947) and co-workers are in the process of publishing[6] a comprehensive series of papers on the effects of routine DDT larviciding on wildlife. In the first paper, concerning surface organisms, it is reported that routine hand applications of DDT dusts cause little apparent damage to surface organisms, nor are the seasonal trends of the various ones affected. DDT fuel oil treatments with dosages as low as 0.025 pound per acre give significant kills of the large surface insects in individual sprayings. Proportionately heavier tolls of these fish food organisms occur with the use of 0.05 and 0.1 pound of DDT per acre. Tarzwell (1947) notes further: "The seasonal effects of routine DDT treatments, as indicated by a comparison of the population of surface organisms in the treated and check ponds, were quite marked. Insects as a group decreased in number. There is (probably) some reduction in the available supply of fish food." This study also indicates significant differences in the kills produced by the use of various solvents.

Plants in general do not seem to be harmed by DDT larviciding (Knowles, et al., 1945; Wasicky, et al., 1944; Wisecup, et al., 1946).

(*References: 15, 17, 26, 27, 28, 29, 30, 38, 40, 49, 50, 51, 52, 61, 65, 82, 97, 113, 124, 130.*)

### DDT LARVICIDING IN RELATION TO TYPES OF BREEDING AREAS

DDT larviciding fits remarkably well the demands placed upon the technique as far as different types of mosquito breeding habitats are concerned. The environmental factors were carefully studied during the early testing period to insure that DDT performed adequately under a wide range of conditions. In clean-surfaced waters with typical grassy edges there is no great problem to consider, but under situations of dirty or foul waters (Hurlbut, et al., 1945) more attention must be given larvicide distribution. The inclusion of spreading agents in the oil-mist tech-

[6] The following studies are being published in Public Health Reports as contributions from Communicable Disease Center, Technical Development Division, Savannah, Georgia:

Erickson, A. B.: Effects of DDT mosquito larviciding on wildlife. Part II: Effects of routine airplane larviciding on bird and mammal populations (Public Health Rep. **62:** 1254–1262, 1947).

Bishop, E. L., Jr.: Effects of DDT mosquito larviciding on wildlife. Part III: The effect on the plankton population of routine larviciding with DDT (Public Health Rep. **62:** 1263–1268, 1947).

Scudder, H. I.: Effects of DDT mosquito larviciding on wildlife. Part IV: Effects on the terrestrial insect population of routine airplane larviciding with DDT. (Unpublished)

nique may aid in obtaining better distribution and kill under adverse conditions. Use of DDT in regions with dense vegetation has been pondered (Cambournac, et al., 1944; Hurlbut, et al., 1945; Wisecup, et al., 1945(b)).

Because of the excellent ability of DDT oil-mists to penetrate dense vegetation there is no need for formerly used extensive clearing operations in the neighborhood of the mosquito breeding area. Simple access trails around the watered area will suffice (Anon., 1946(b)). Application of DDT larviciding to still waters (Ribbands, 1945) is simple in comparison to flowing waters (Deonier, et al., 1945(a); Johnson, 1946; Ribbands, 1945; Wisecup, et al., 1946) which pose questions, many of which are unanswered as yet. Wisecup, et al. (1946), has studied the possible effects of excessive aeration upon DDT larviciding.

Normally, rains within about 30 minutes following treatments are not detrimental. Deonier, et al. (1945(b)), have observed the effects of artificially produced rains upon the effective kill, while Lever (1944) has noted the suitability of DDT as a mosquito larvicide in areas having an annual rainfall of as much as 120 inches. Elevation of temperature does not seem to affect larviciding, yet the authors have noted at times that cold weather seems to increase the killing power of DDT larvicides.

## DDT LARVICIDE DISPERSION

A great factor in the successful use of DDT as a mosquito larvicide is the extent to which its general physical and chemical properties (Deonier, et al., 1945(b); Haller, et al., 1945; Lindquist, et al., 1945(b)) lend themselves to the demands of the methods used. Deonier (1945(b)) noted in an early paper: "The physical properties of DDT make it very adaptable for use as a larvicide. It is soluble in a wide range of organic solvents. Its solubility in the petroleum oils makes possible wide application in mosquito-control programs where larvicidal oils are now used. DDT has a specific gravity of 1.6 and is practically insoluble in water. It is also non-wetting and therefore resistant to sinking by rains. When applied as a larvicide it appears to be stable chemically to the action of sunlight and water."

DDT may be successfully applied as a dust in combination with certain diluents, as a solution, in a colloidal suspension, or as an emulsion. It apparently has no fumigant action.

DDT dust diluents have been studied to some extent; Jones and co-workers (1945-(a)) in early tests considered the problem of non-wetting diluents, while most workers have indicated that pyrophyllite is an adequate carrier (Deonier, et al., 1945(a); 1945(c); Eide, et al., 1945(b); Ginsburg, 1945(a); 1945(c); Henderson, et al., 1946; Horsfall, 1946; Jones, et al., 1945(a); Knipling, 1945(b); Mathis, et al., 1947; Metcalf, et al., 1945; Tarzwell, 1947; Wilson, et al., 1946; Yates, 1946). The authors have shown a preference for an especially prepared calcium carbonate as a dust diluent. Lime is not used since it is said to hasten the deterioration of DDT.

A host of DDT solvents and auxiliary solvents have been tested, including most of those mentioned in standard solvency tables. Some of the more commonly used ones have been petroleum oils (Diesel oil, Number 2 fuel oil, waste crank case oil, kerosene) coal gas distillate, xylene, acetone, cyclohexanone and various alcohols. Specialized high-boiling-point oily solvents are used in thermo-aerosol disperson from

airplanes (Kruse, et al., 1946). More information is needed as to the importance of the relative volatility of various solvents. The effectiveness of the solvent used alone has interested some workers (Arnold, et al., 1945; Ferguson, et al., 1947; Tarzwell, 1947).

Emulsifying agents used have been those calculated to give stable larvicides (Arnold, et al., 1945; Buxton, 1945(a); Ginsburg, 1945(b); Jones, et al., 1946(a); 1946(b); Travis, et al., 1946; Wisecup, et al., 1945(a)). Emulsifying-spreading agents have been included in certain recommended DDT larvicidal formulations (Ferguson, et al., 1947). The spreading agents are thought to improve the effectiveness of DDT oil mists by increasing the spread of the solution on the water surface.

The surface film of oil with the incorporation of DDT appears to be the most usable form of mosquito larvicide (Arnold, et al., 1945; Barber, 1945; Buxton, 1945(a); Deonier, et al., 1945(a); Mandekos, 1944; Mathis, et al., 1947; Ribbands, 1945; Simmons, J. S., 1945(d); Stage, 1943; Stilson, 1946; Tarzwell, 1947; Travis, et al., 1946; Wisecup, et al., 1946).

The surface film may be obtained by a number of methods of dispersion. The use of drip cans has not been a notably successful method (Deonier, et al., 1945(a); Wisecup, et al., 1945(c)) yet with mechanical improvements it should be useful in larval control in running waters. Other improvised techniques of doubtful value are the pouring of DDT oil solutions (Anon., 1945(c); Wisecup, et al., 1945(c)) and the broadcasting of oil soaked sawdust upon the water surface (Anon, 1945(c); Wisecup, et al., 1945(c))[7]. Possibly the most effective manual method of producing the surface film at present is the DDT oil-mist technique (Ferguson, et al., 1947; Mathis, et al., 1947).

The aerosol method of larvicide dispersion from the ground (Goodhue, 1944; 1946; Goodhue, et al., 1942(b); Johnstone, et al., 1945) is limited to specific categories. In general its use is confined to the larger, more inaccessible mosquito breeding areas or to roadside ditches. The *Jeep* exhaust aerosol (Rice, et al., 1946) is produced by breaking up high-boiling-point oils into fine particles by the action of the expelled motor gases in the venturi of the exhaust line. It is a method with limited use.

The steam-oil solution type of aerosol dispersal has been used (Bresica, et al., 1946; Glasgow, et al., 1946; Goodhue, 1946). The equipment, though bulky, may be carried by truck or moved along shorelines in the spreading of the cloud of insecticide.

The pre-flooding dust treatment has been established as promising in the control of mosquitoes, especially psorophorans, in the rice field regions (Horsfall, 1946; Wisecup, et al., 1945(a); 1945(c)). High dosages are necessary with the method. Another interesting technique reported is that of controlling larvae in flowing streams by means of slowly disintegrating pellets of paradichlorobenzene which act as a vehicle for DDT (Johnson, 1946).

The original liquified-gas type of aerosol bomb has been used but little in experimental larviciding tests (Goodhue, 1942(a); Goodhue, et al., 1942(b); Jones, et al., 1945(c)). There is nothing to recommend the method except its lighter weight.

[7] Also Smith, Howard F. and Francisco J. Dy: The use of DDT-treated sawdust for the control of anopheline mosquito larvae in streams. Acta Medica Philippina 4(2): Oct-Dec. 1947.

Smoke grenades, productive of dense clouds of extremely fine particles, have been tested in preliminary trials (Deonier, et al., 1945(a)).

Much work has been done with airplane distribution of DDT larvicides (Anon., 1946(c); 1945(c); Deonier, et al., 1945(a); 1945(c); Knipling, 1945(b); 1946; Kruse, et al., 1946; Lindquist, et al., 1945(a); Logue, et al., 1945; McCormick, 1944; Metcalf, et al., 1945; Schreuder, et al., 1945; Sebora, et al., 1946; Stilson, 1946; Travis, et al., 1946; Wisecup, et al., 1945(a); Yuill, et al., 1946; Yust, 1946). Two general methods are used; the exhaust line type of thermo-aerosol (as in the *Jeep*, Rice, et al., 1946,) and the oil-mist method. With the exhaust venturi type of expulsion special non-corrosive solvents which have relatively high boiling points and high solvency for DDT are indicated for use. Airplane distribution is developed to serve large or inaccessible areas, or to serve in emergencies such as in the control of mosquito breeding in overflow regions in the recent Mississippi River flood. It is used routinely on some control programs (Kruse, et al., 1946): "The use of the airplane exhaust generator for the production of DDT aerosols employed in the control of *Anopheles quadrimaculatus* larvae has been extensively studied in the last two years. This type of equipment is ideally suited for larvicidal operations on the impounded waters of the Tennessee Valley Authority as it provides a relatively uniform coverage over wide swaths at exceedingly low rates of discharge. The particle size of the larvicide generated may be controlled to meet field requirements. The use of particles of aerosol size will result in the effective penetration of heavy vegetative cover. The visible smoke cloud produced serves as a marker to guide the pilot. The equipment is simple, inexpensive and easily installed on the 450-hp *4DX* and 220-hp *PT-17 Stearman* biplanes which were available for study." In such control operations the penetration ability of the droplets into woody and herbaceous types of water covering is an important factor. Kruse (1946) further indicates that one must use particles under 50 microns in diameter in order to insure control in dense vegetation. Generally a size range of 25–50 microns is recommended. In his analysis of the swath cross-section, Kruse notes that only a dosage of 0.001 pound per acre exists 100 feet from the center line of flight, also that only about 9 per cent of the DDT discharged is recovered in analytical surveys. Low speed airplanes using a dispersion swath of from 150–300 feet are employed for this method.

Use of the aerosol method has given impetus to the development of several techniques for larvicide particle size analysis (Bishopp, 1946(a); Deonier, et al., 1945(c); Jones, et al., 1945(b); Knipling, 1946; Kruse, et al., 1946; Sebora, et al., 1946). Those successfully used have been the analysis of droplet size on plain and carbon-covered slides placed in the zone of operation, the use of the impingement apparatus for gathering droplets for size study and the quantitative recovery of DDT from glass plates. These have been supplemented by studying the kills on larvae placed in pans of water in the treatment zone, or upon natural larval populations.

Possibly no other insecticide ever studied has had its success so profoundly based on the development of proper dispersion equipment. In all phases of DDT larviciding (manual, power, and airplane) there have been noteworthy contributions in the field of equipment (Anon., 1946(b); 1947; 1946(c); 1945(b); Deonier, et al., 1945(c); Eide, et al., 1945(b); Farr, 1945; Ferguson, et al., 1947; Knipling, 1946; Kruse, et al.,

1946; Logue, et al., 1945; Mathis, et al., 1947; Metcalf, et al., 1945; Rice, et al., 1946; Sebora, et al., 1946; Wisecup, et al., 1945(a); 1945(b)). While it is true that DDT larvicide is reasonably effective when dispersed from an ordinary domestic fly spray gun, results are strikingly more effective if proper apparatus is used. The present tendency with manual equipment is toward lightweight low volume sprayers and dusters; the latter especially needs improvement. Vehicle and boat drawn power equipment is also being adapted to the needs of DDT larviciding.

(*References: 15, 17, 31, 39, 50, 59, 67, 70, 71, 72, 78, 81, 85, 86, 118, 119, 124, 133.*)

### MILITARY USAGE OF DDT LARVICIDES

The civilian use of DDT mosquito larvicides is to a great extent the result of military needs during the war. Only a small part of the literature on the military aspects of DDT larviciding has appeared in print. McCoy (1944(b)) sagely observes: "The war against insects requires the same elements as a military campaign. New chemicals effective against insects have value comparable to the secret weapons of real warfare." Deonier in an early paper (1945(a)) predicted a widespread use by military control personnel of the DDT oil film method: "Oil solutions will be used by the Armed Forces much more widely than dusts because of the ease of applicaion, availability of carrier (solvent) and adaptability to existing equipment."

Jones, et al. (1945(b)), have reported on tests on which the use of DDT larvicides from fast combat aircraft was based. Further observations on the use of the slower *Cub* type of airplane as an adjunct to manual ground techniques has been given by Travis, et al. (1946). Preliminary tests with the *HNS-1* helicopter as a larvicide vehicle have shown a number of disadvantages with this method (Yuill, et al., 1946).

The reports of Dews and Morrill (1946) are presented as an example of the use of DDT larvicides at an Army stateside training installation. A description of larviciding operations during an invasion of a Pacific island by a Marine division is of interest (Logue, et al., 1945). Pre-invasion larval control methods were instituted at Okinawa (Stilson, 1946).

(*References: 30, 31, 36, 43, 46, 58, 64, 74, 77, 87, 94, 95, 96, 111, 112, 123, 125, 132, 134, 138, 139.*)

### ECONOMICS OF DDT LARVICIDING

This literature indicates that significant savings may be made in the use of all forms of DDT larvicides (exclusive of dusts) in comparison to previously empoyed methods. The responsible factors are the relatively high potency, ease of application, adaptability of the material and low cost of larvicide components. Mathis, Ferguson and Simmons (1947) have shown that in control operations approximately the same time is required to treat breeding areas of 10 feet or less in width, regardless of whether paris green or DDT dust is used. However, the DDT oil-mist technique (1 gallon per acre) gives a time saving of 36 per cent as compared with dusts in general. The DDT oil-water emulsion (15 gallons per acre) requires more time to apply than either of the dusts or the oil-mist. In breeding areas greater than 10 feet in width (ponds, swamps, etc.), a 52 per cent saving was obtained over either of the dusts by the use of the DDT oil-mist formulation. While the 1-gallon-per-acre DDT oil-mist technique

is obviously more economical than the 15-gallon-per-acre DDT oil-water emulsion method, there is no significant difference in the efficiency of the treatments based on applications of the same DDT dosage rate. The pay load factor in airplanes had impeded the wider use of larvicides applied by airplanes before the advent of DDT concentrates (Deonier, et al., 1945(c)). Kruse and Metcalf (1946) in their analysis of the airplane dispersion of DDT note: "Comparative cost records of DDT larviciding operation and paris green dusting indicate an approximate cost per acre of $0.26 for DDT and $0.79 for paris green, and the DDT treatments resulted in more effective anopheline larvae control." Savings are realized even though relatively high DDT dosages (up to 0.3 pound per acre) are employed. Dews and Morrill (1946) have noted a drop from 23 gallons of oil per acre to 8.67 gallons per acre with the incorporation of DDT. In one representative Army post they report a labor expenditure of 2.9 man-hours per acre for the dispersion of over 15,000 gallons of DDT oil solution at 3.7 gallons per acre.

It has been strongly emphasized (Anon., 1946(b); Henderson, et al., 1946; Mathis, et al., 1947; Wisecup, et al., 1945(b)), that because of the more exacting nature of the newer DDT larviciding techniques intelligent control personnel amenable to training is a necessity.

(*References: 10, 36, 39, 45, 61, 81, 93, 133.*)

## DISCUSSION

Sound and rapid progress has been made in the efforts to correlate and coordinate mosquito larviciding with adult mosquito sanitation. Gahan and Lindquist (1945) have shown possibilities of significant reductions in larval counts in rice fields adjacent to test areas in which all buildings were subjected to DDT residual wall sprays. There is some evidence (Anon., 1946(a)) that continued use of the DDT oil-mist treatment may reduce the adult mosquito counts in the immediate area. Perhaps it is not over optimistic to visualize the future development of a technique in which a few treatments per year will insure an area against significant mosquito production.

The field of mosquito larviciding has definite need for more information on the effective swath width for the various formulations and methods. The literature affords but scant information on the classic types of formerly used larvicides in this respect.

Many large mosquito control projects have a definite need for airplane dispersion of DDT or other compounds. Less expensive solvents and simple light-weight easily-detachable dispersion equipment which would increase both the effective swath width and pilot safety are certainly factors to be considered. Data as to relative effectiveness of airplane thermo-aerosol and mechanical atomization of oily solutions, as well as other problems of airplane application, have been discussed in a conference called by the American Mosquito Control Association (Russell, 1948).

Since there is significant variation in the effectiveness of DDT in various solvents (Upholt, 1947), and since it is more effective in several solvents than it is in the presently recommended kerosene or Number 2 fuel oil, there is a reasonable demand for research in this respect.

The development of proper dust dispersion equipment is a deterrent in seeking

needed data on the relative value of DDT dust and liquid larvicides. DDT dusts might become especially useful in areas having wildlife value.

Future developments in the field of DDT liquid larviciding may be chiefly centered in basic studies on the type of solvent and solvent spreading agent and continued efforts to improve dispersion equipment.

## SUMMARY

This paper presents information upon the use of DDT in mosquito larviciding reviewed principally from the control or operational viewpoint. The appended bibliography cannot be complete because of the serious deletion noted above of the data consigned to restricted files.

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# PUPAE OF THE NEARCTIC ANOPHELINE MOSQUITOES NORTH OF MEXICO[1]

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In view of the attention that has been centered on nearly all phases of the life history, taxonomy and control of Nearctic anopheline mosquitoes it is extraordinary that the pupal stage of most species has been almost completely neglected. Only five of the fifteen species have been more or less completely described in the pupal stage. The purpose of this study, therefore, is to describe (or redescribe), and give illustrations of the anopheline pupae of the Nearctic region north of Mexico and to present a tentative key for their identification.

The system of chaetotaxy used by the writer in a previous paper on mosquito pupae (Penn, 1949) has been made obsolete by the comparative study of Knight and Chamberlain (1948). The system proposed by them has been adopted here and is followed without change.

It is a pleasure to acknowledge with thanks the cooperation of the following in obtaining pupae of certain species for this study: Dr. Robert Matheson and Mr. Richard F. Darsie, Jr. of Cornell University; Dr. Alan Stone of the U. S. Bureau of Entomology and Plant Quarantine; Dr. Richard M. Bohart of the University of California; Dr. Luis Vargas of the Mexican Instituto de Salubridad y Enfermedades Tropicales; and Drs. Thomas F. Hall and W. E. Snow of the Tennessee Valley Authority.

Abbreviations found in the text of the paper have the following equivalents. Location of specimens examined: *CU* = Cornell University; *TU* = Tulane University; and, *USNM* = U. S. National Museum; collectors: GHB = George H. Bick; GHP = George H. Penn; JVB = John V. Baus; LLE = Leslie L. Ellis; MHP = Mimi H. Penn; RFD = Richard F. Darsie, Jr.; RM = Robert Matheson; RMB = Richard M. Bohart; and WVK = Willard V. King.

## TENTATIVE KEY TO THE PUPAE OF NEARCTIC ANOPHELES

1. Seta *8*-VIII with at least five smaller side branches..... 2
   Seta *8*-VIII without side branches, either acute or shallowly cleft at tip.... 13
2. Paddle margin with conspicuous, closely spaced, coarse, wide teeth laterally. (Figure 1)..... *A. walkeri*
   Paddle margin various, but never with closely spaced, coarse teeth..... 3
3. Seta *8*-VII long and thin, gradually tapering to an acute point; seta *8*-V at least seven times longer than its diameter..... 4
   Seta *8*-VII short and stout, usually with blunt apex, or, if with sharp apex,

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not gradually tapering; seta *8*-V never more than five times longer than its diameter..... 6

4. Seta *8*-VII more than ten times longer than its diameter; seta *8*-VIII with less than 9 side branches; seta *2*-II less than 3-forked; seta *2*-III less than 5-forked; seta *2*-IV and *5*-IV less than 3-forked; seta *2*-V simple or 2-forked; seta *2*-VI always simple; seta *5*-III less than 7-forked. (Figure 2). *A. occidentalis*

Seta *8*-VII about seven times longer than its diameter; seta *8*-VIII with more than 12 side branches; seta *2*-II more than *5*-forked; seta *2*-III more than 6-forked; seta *2*-IV and *5*-IV more than 5-forked; seta *2*-V more than 3-forked; seta *2*-VI more than 2-forked; seta *5*-III more than 8-forked..... 5

5. Terminal paddle seta (*8*) 2-branched; seta *5*-IV less than 8-forked; seta *3*-I less than 4-forked; generally found only west of the Rocky Mountains. (Figure 3)..... *A. freeborni*

Terminal paddle seta (*8*) always simple; seta *5*-IV more than 9-forked; seta *3*-I more than 6-forked; generally found only east of the Rocky Mountains. (Figure 10)..... *A. crucians*

6. Terminal paddle seta (*8*) usually with 3 or 4 smaller side branches (occasionally only one side branch, but seta never simple)..... 7

Terminal paddle seta (*8*) always simple..... 8

7. Setae *7* and *10* on segment II about equal in length; seta *5* more than 7-forked on segments III and IV, less than 3-forked on segments V and VI, and more than 3-forked on VIII; seta *2*-IV more than 6-forked. (Figure 4) *A. quadrimaculatus*

Seta *7*-II at least twice the length of *10*-II; seta *5* less than 6-forked on segments III and IV, more than 4-forked on segments V and VI, and not more than 2-forked on VIII; seta *2*-IV less than 5-forked. (Figure 5).. *A. atropos*

8. Seta *7*-II at least three times the length of *10*-II. (Figure 6)..... *A. aztecus*

Seta *7*-II more or less equal in length to *10*-II, or, seta *7*-II not more than twice the length of *10*-II..... 9

9. Seta *8*-IV averaging about half or less the length of *8*-VII..... 10

Seta *8*-IV averaging two-thirds or more the length of *8*-VII..... 11

10. Seta *4*-IV simple; seta *5*-IV less than 6-forked; seta *6*-IV more than 5-forked; seta *7*-VII simple; cephalothoracic setae: *7* more than 2-forked, *10* simple, and *11* more than 3-forked. (Figure 7)..... *A. punctipennis*

Seta *4*-IV more than 2-forked; seta *5*-IV more than 6-forked; seta *6*-IV less than 4-forked; seta *7*-VII more than 3-forked; cephalothoracic setae: *7* simple, *10* more than 2-forked, and *11* less than 2-forked. (Figure 8) *A. earlei*

11. Seta *5* more than 13-forked on segment IV, more than 9-forked on V, more than 8-forked on VI, and more than 7-forked on VII; seta *4*-II more than 3-forked near tip. (Figure 9)..... *A. georgianus*

Seta *5* less than 11-forked on segment IV, less than 8-forked on V, less than 7-forked on VI, and less than 5-forked on VII; seta *4*-II usually simple, and never more than 2-forked near tip..... 12

12. Seta *5*-III more than 8-forked; seta *3*-I more than 6-forked; seta *4*-I usually 2-forked or simple; metanotal seta *10* simple. (Figure 10)..... *A. crucians*
    Seta *5*-III less than 7-forked; seta *3*-I less than 4-forked; seta *4*-I more than 2-forked; metanotal seta *10* more than 2-forked. (Figure 11).... *A. bradleyi*
13. Setae *8*-VII and VIII approximately twice the length of *8*-VI; seta *5*-III very small, simple; seta *2* on segments VI and VII very small, less than one-tenth the length of the respective segments. (Figure 12)..... *A. barberi*
    Setae *8*-VI, VII and VIII approximately same length; seta *5*-III medium to long, four- or more forked; seta *2* on segments VI and VII very long, exceeding lengths of the respective segments..... 14
14. Seta *5*-I long, four to five times longer than *6*-I; seta *7*-II nearly twice the length of *10*-II. (Figure 13)..... *A. franciscanus*
    Seta *5*-I small, sub-equal to *6*-I; seta *7*-II less than the length of *10*-II. (Figure 14)..... *A. albimanus*

ANOPHELES (ANOPHELES) WALKERI Theobald (1901)

Pupa heretofore undescribed. However, Hurlbut (1941) gave a few general notes on the species; Burgess (1946) described the dorsal pigmentation of living pupae; Sabrosky (1946) included the species in a key to the pupae of Michigan anophelines; and, Vargas and Matheson (1948) included it in a key to the pupae of the *maculipennis* complex of North America.

*Specimens Examined.* Seven pupal exuviae as follows: NEW YORK: 1 ♀ from Cayuta Lake, Schuyler County, VI-14-46, GHP, GHB (*TU 37*). TENNESSEE: 2 ♂♂ and 4 ♀♀ from Reelfoot Lake, Obion County, IX-10-39, G. E. Quinby (*USNM*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae all small or medium and rather inconspicuous except seta *7* which is long and simple. Metanotal setae as follows: *10* medium, simple; *11* medium, three- to four-forked; *12* long, three-forked.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* medium, simple or rarely three-forked; *4* medium, four- to two-forked; *5* long, two-forked near tip, range from simple to three-forked; *6* medium, five- to six-forked; *7* medium-long, three-forked near middle, or rarely simple; *8* small, three-forked to simple; *10* medium, five- to six-forked.

*Segment II: 1* very small, simple; *2* medium, six- to seven-forked; *3* medium, six- to seven-forked; *4* medium, stout, simple to two-forked near tip; *5* small, five- or six-forked; *6* small, four- to six-forked; *7* small, three- or four-forked; *8* small, transparent spine; *10* medium, five-forked, or rarely four-forked.

*Segment III: 1* very small, simple or two-forked; *2* medium, five- or six-forked; setae *3*, *4* and *6* inconspicuous on this and segments IV through VII; *5* medium, five- to seven-forked; *7* medium, five- to three-forked; *8* small, stout, dark-pigmented spine about three times the length of *8*-II.

*Segment IV: 1* very small, simple on this and all following segments; *2* medium-long, five- or six-forked; *5* medium, five-forked, or rarely ten-forked; *7* medium, three- or four-forked; *8* similar to and about twice the length of *8*-III.

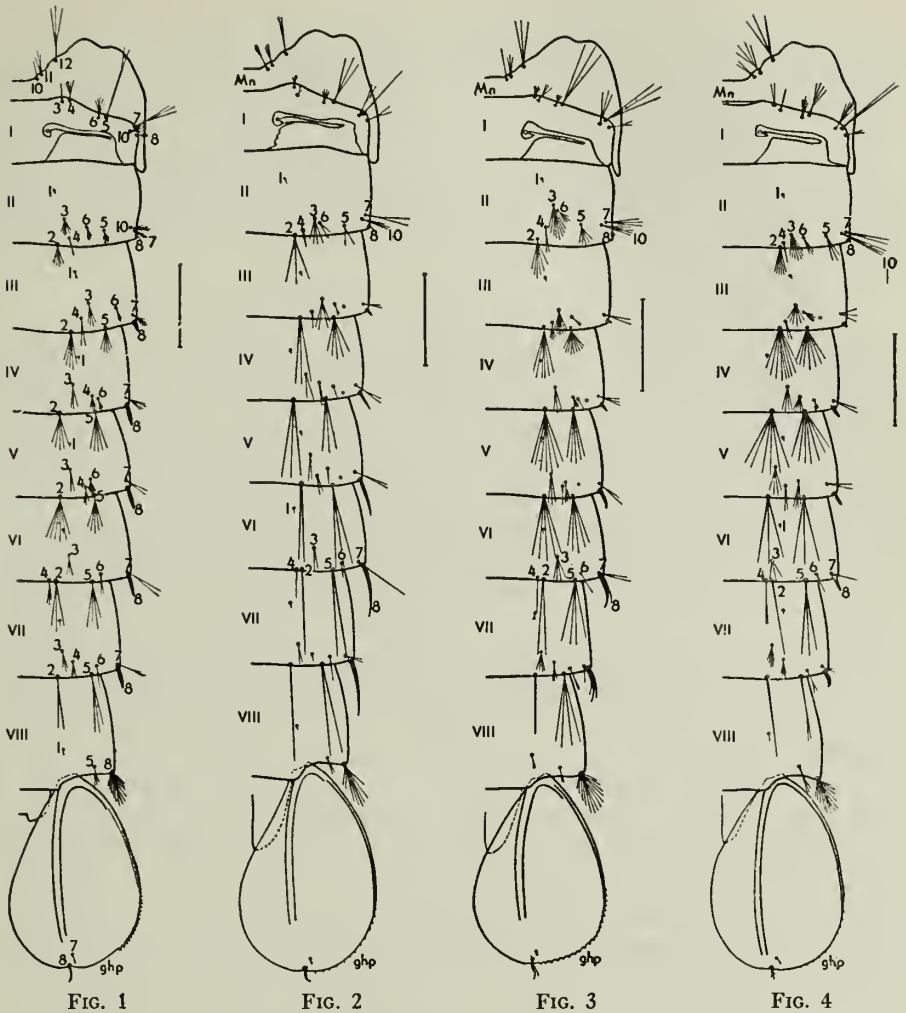


FIG. 1 FIG. 2 FIG. 3 FIG. 4

FIG. 1. *Anopheles walkeri.* Right dorsal aspect of metanotum and abdomen of female from Tennessee.[2]

FIG. 2. *Anopheles occidentalis.* Right dorsal aspect of metanotum and abdomen of male from California.

FIG. 3. *Anopheles freeborni.* Right dorsal aspect of metanotum and abdomen of male from California.

FIG. 4. *Anopheles quadrimaculatus.* Right dorsal aspect of metanotum and abdomen of male from Louisiana.

*Segment V: 2* long, six- to four-forked; *5* medium-long, five- to four-forked; *7* medium, two- to four-forked; *8* similar to and averaging slightly longer than *8*-IV.

*Segment VI: 2* long, three- or four-forked; *5* long, four- to six-forked; *7* medium-long, two- to four-forked; *8* similar to and about equal to *8*-V.

[2] Vertical bar to right of this and other figures equals 0.5 mm.

*Segment VII: 2* long, two-forked; *5* long, three- to four-forked; *7* medium, three-forked near tip; *8* similar to and averaging slightly shorter than *8*-VI.

*Segment VIII: 5* small, three-forked, or rarely two- or four-forked; *8* dark, basally about same length as *8*-VII with twelve to sixteen smaller side branches.

*Paddle:* Buttress broad at base, giving way almost immediately to a series of short, coarse, blunt teeth which increase gradually in size nearly to apex; remainder of margin either entire, or with a few scattered short, fine hairs around apex. Accessory seta (*7*) very small, two- to three-forked; terminal seta (*8*) medium, stout, simple or rarely split at tip.

ANOPHELES (ANOPHELES) OCCIDENTALIS Dyar and Knab (1906)

Pupa incompletely described by Aitken (1945) who included a figure of the terminal abdominal segments, and by Vargas (1943) who included a complete figure of the metanotum and abdominal segments; Vargas and Matheson (1948) included the species in a key to the Nearctic pupae of the *maculipennis* complex and gave figures of the metanotum, abdomen and trumpet of specimens from California. Burgess (1946) gave a brief description of the dorsal pigmentation of living pupae.

*Specimens Examined.* Three pupal exuviae as follows: CALIFORNIA: 1 ♂ and 2 ♀ ♀ from 10 mi. s. San Francisco, San Francisco County, IX-17-47, RMB (*TU 706*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae small to medium except seta *7* which is rather long and simple. Metanotal setae as follows: *10* medium, three-forked to simple; *11* small, two- to three-forked; *12* long, two- to three-forked near middle.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* small, two-forked, or simple to three-forked; *4* small, two-forked, or simple to four-forked; *5* long, three- to two-forked; *6* small, five- to seven-forked; *7* long, simple or two-forked; *8* medium, simple or two-forked near middle; *10* medium, two- to four-forked.

*Segment II: 1* very small, simple on this and all following segments; *2* conspicuous, medium-long, three- to two-forked; *3* medium, four- to seven-forked; *4* small, stout, simple or two-forked at tip; *5* small, three- to two-forked; *6* small, two- to six-forked; *7* medium-long, two-forked; *8* small, thin, transparent; *10* medium, three-forked.

*Segment III:* Setae *3*, *4* and *6* rather inconspicuous, small to medium and variously forked on this and segments IV through VII; *2* long, three- to five-forked; *5* medium-long, five-forked, range from three- to seven-forked; *7* small, two-forked; *8* small, stout, transparent and roughly equal to or very slightly longer than *8*-II.

*Segment IV: 2* long, three- to two-forked; *5* long, three- to two-forked; *7* medium, longer than *8*, two-forked or simple; *8* dark-pigmented, tapered to an acute point, roughly twice the length of *8*-III.

*Segment V: 2* long, two-forked or simple; *5* long, three- to two-forked; *7* medium, about equal to *8*, two-forked or simple; *8* similar to and roughly twice the length of *8*-IV.

*Segment VI: 2* long, simple; *5* long, two-forked; *7* long, roughly equal to *8*, simple; *8* similar to and varying from a half longer to twice the length of *8*-V.

*Segment VII: 2* long, simple; *5* long, two-forked or simple; *7* small, less than one-

fourth the length of *8*, simple or two-forked; *8* similar to and averaging a third longer than *8*-VI.

*Segment VIII: 5* small, two-forked; *8* shorter than *8*-VII, with six to eight smaller side branches.

*Paddle:* Apical half of lateral margin with a few widely spaced fine teeth. Accessory seta (*7*) very small, two-forked or simple; terminal seta (*8*) small, stout, simple and slightly curved.

### ANOPHELES (ANOPHELES) FREEBORNI Aitken (1939)

Pupa incompletely described by Aitken (1945) who included a figure of the terminal abdominal segments; Burgess (1946) presented a photograph of the dorsum of living pupae and included a few notes on the pigmentation.

*Specimens Examined.* Three pupal exuviae as follows: CALIFORNIA: 2 ♂♂ and 1 ♀ from Davis, Yolo County, VIII-?-46, RMB (*TU 703*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae all medium in size and rather inconspicuous except seta *7* which is long and simple. Metanotal setae as follows: *10* medium, simple or two-forked near tip; *11* small, three-forked, range from two- to four-forked; *12* long, three- or two-forked.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* small, four- or three-forked; *4* small, three-forked, range from two- to four-forked; *5* long, three- or two-forked; *6* small, four- or five-forked; *7* long, three- or two-forked; *8* medium, simple to three-forked; *10* medium, two- to four-forked.

*Segment II: 1* small, simple seta on this and all following segments; *2* medium, five- to seven-forked; *3* medium, seven- to ten-forked; *4* medium, two-forked or simple; *5* medium, four- or five-forked; *6* small, two- to six-forked; *7* medium, three- to five-forked; *8* small, non-pigmented spine; *10* two- to four-forked, subequal to *7* in length.

*Segment III:* Setae *3*, *4* and *6* rather small and unimportant on this and segments IV through VII; *2* long, six- to eight-forked; *5* medium, nine- to eleven-forked; *7* medium, three- or two-forked; *8* small, light-brown pigmented spine subequal to *8*-II in length.

*Segment IV: 2* long, seven- to five-forked; *5* long, five- to eight-forked; *7* medium, at least twice the length of *8*, two- or three-forked; *8* similar to and two to three times the length of *8*-III.

*Segment V: 2* long, five- to three-forked; *5* long, five- to three-forked; *7* medium, slightly longer than *8*, two-forked, range from simple to three-forked; *8* one-third longer to twice the length of *8*-IV, dark-pigmented, slightly curved, and with one or two small side branches on some specimens.

*Segment VI: 2* long, two-forked; *5* long, four-forked, or two- to three-forked; *7* medium, subequal to *8*, simple or two-forked; *8* dark-pigmented, well curved inwards, sometimes with smaller side branches, nearly twice the length of *8*-V.

*Segment VII: 2* long, simple or two-forked; *5* long, four-forked, or two- to three-forked; *7* small, less than half the length of *8*, two-forked, or three- to four-forked; *8* similar and subequal to *8*-VI.

*Segment VIII:* *5* small, two- to three-forked; *8* shorter than *8*-VII, dark-pigmented, with fourteen to thirteen smaller side branches.

*Paddle:* Distal half of lateral margin with widely spaced fine teeth. Accessory seta (*7*) very small, simple or two-forked; terminal seta (*8*) small, stout, slightly curved, two-branched.

ANOPHELES (ANOPHELES) QUADRIMACULATUS (Say, 1824)

Pupa heretofore undescribed although Vargas (1943) included a figure of the metanotum and dorsal abdominal segments without a description; Aitken (1945) included a few notes on pupae from Jefferson County, Florida; Burgess (1946) described the pigmentation of the dorsum of living pupae and included a photograph; Sabrosky (1946) included the species in a key to the pupae of Michigan anophelines; and, Vargas and Matheson (1948) included it in a key to the pupae of the *maculipennis* complex in North America.

*Specimens Examined.* Twenty-five pupal exuviae as follows: LOUISIANA: 1 ♂ from Chalmette, St. Bernard Parish, XI-7-47, GHP, LLE, JVB (*TU 125*); 2 ♂♂ and 6♀ ♀ from Robert, Tangipahoa Parish, VII-31-48, GHP, MHP (*TU 717*); 1 ♀ from 1 mi. s. Covington, St. Tammany Parish, VIII-16-48, GHP, MHP (*TU 793*); 2 ♂♂ and 2 ♀ ♀ from 1.5 mi. n. Madisonville, St. Tammany Parish, VIII-16-48, GHP, MHP (*TU 794*); 1 ♂ and 2 ♀ ♀ from 3 mi. n. Franklin, St. Mary Parish, VIII-25-48, GHP, MHP (*TU 105*). SOUTH CAROLINA: 1 ♂ from Parris Island, Beaufort County, VI-19-45, GHB (*TU P-488*); 5 ♂♂ and 2 ♀ ♀ from Parris Island, Beaufort County, VII-23-45, GHB (*TU P-509*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae all medium or small and unimportant except seta *7* which is long and simple. Metanotal setae as follows: *10* medium, two-forked near tip, range from simple to three-forked; *11* medium, three- or four-forked; *12* long, usually three-forked, range from two- to five-forked.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* small, three- to five-forked, or rarely two-forked; *4* small, three-forked, or occasionally simple or two-forked; *5* medium, five- to three-forked; *6* small, five- to nine-forked; *7* long, two- to four-forked; *8* small, two-forked or simple, or rarely four-forked; *10* medium, five- or six-forked, or ccasionally two-forked.

*Segment II: 1* very small, simple on this and all following segments; *2* medium, six- to four-forked; *3* medium, six- to eight-forked; *4* small, simple or two-forked near tip; *5* medium, three- or four-forked; *6* small, five- to three-forked; *7* long, two- to four-forked; *8* small, transparent peg; *10* long, two- to five-forked.

*Segment III:* Setae *3*, *4* and *6* rather inconspicuous, small to medium on this and segments IV through VII; *2* long, seven- to nine-forked; *5* medium, seven- to eleven-forked; *7* small, two- to four-forked; *8* small, dark-pigmented peg about twice the length of *8*-II.

*Segment IV: 2* long, six- to eight-forked; *5* long, seven- to eight-forked; *7* medium, two- to three-forked; *8* similar to and almost twice the length of *8*-III.

*Segment V: 2* long, two- to three-forked, or rarely simple; *5* long, three- to two-forked; *7* medium, two- to three-forked; *8* similar to and slightly longer than *8*-IV.

*Segment VI: 2* long, simple; *5* long, three- or two-forked; *7* medium, simple; *8* similar to and slightly longer than *8*-V.

*Segment VII: 2* long, simple; *5* long, two- or three-forked near tip, rarely simple; *7* small, simple or two-forked; *8* similar to and slightly longer than *8*-VI.

*Segment VIII: 5* medium, three-forked, or rarely four-forked; *8* with stout, dark base and ten to sixteen smaller side branches.

*Paddle:* Margin very finely serrate laterally at apex of buttress, from there on with short, widely scattered fine hairs around apical third. Accessory seta (*7*) small, two-forked, or occasionally simple or three-forked; terminal seta (*8*) light brown, medium, usually with one or two smaller side branches, rarely simple, usually straight.

### ANOPHELES (ANOPHELES) ATROPOS Dyar and Knab (1906)

The pupa has not been described previously although Beyer (1923) included a few non-diagnostic lines in a discussion of the species, and Vargas and Matheson (1948) included it in a key to the Nearctic pupae of the *maculipennis* complex.

*Specimens Examined.* Two pupal exuviae as follows: FLORIDA: 2 ♀♀ from the road to St. George, Nassau County, VIII-16-45, A. E. Pritchard Nos. 104, 105 (*USNM*).

*Cephalothorax.* Postocular, dorsal and supra-alar setae absent from both specimens. Anterothoracic: *4* medium, four-forked; *5* medium, five-forked; *6* medium, two-forked; *7* long, simple or two-forked. Metanotal: *10* medium, two-forked; *11* medium, two-forked; *12* medium, three-forked.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* small, simple; *4* small, two- or three-forked; *5* medium, two- or three-forked; *6* missing from both specimens; *7* long, three-forked; *8* small, simple; *10* medium, six- or seven-forked near base.

*Segment II: 1* small, simple on this and all following segments; *2* medium, five- or six-forked; *3* small, four-forked; *4* medium, stout, simple; *5* small, three-forked; *6* small, simple; *7* medium, three- or two-forked; *8* very small, transparent, stout spine; *10* small, three- or four-forked near base.

*Segment III:* Setae *3*, *4* and *6* all small and unimportant on this and segments IV through VII; *2* medium-long, five- to seven-forked; *5* medium, six- or five-forked; *7* medium, three- to six-forked; *8* small, dark-pigmented, stout spine at least twice the length of *8*-II.

*Segment IV: 2* long, four-forked; *5* long, four-forked; *7* medium, two- or three-forked; *8* similar to and about twice the length of *8*-III.

*Segment V: 2* long, two-forked; *5* long, four- to six-forked; *7* medium, two-forked; *8* similar to and about the same length as *8*-IV.

*Segment VI: 2* long, two-forked; *5* long, four- to five-forked; *7* medium, two-forked near apex; *8* similar to and about same length as *8*-V.

*Segment VII: 2* long, simple or two-forked; *5* long, four- or three-forked; *7* small, simple, very inconspicuous; *8* similar to and about one-third longer than *8*-VI.

*Segment VIII: 5* small, two-forked; *8* with stout basal spine about equal to *8*-VII with eight to nine smaller side branches.

*Paddle:* Buttress strong, descending from base two-thirds of way to apex, with a

fringe of small teeth which give way to a fringe of rather inconspicuous short fine hairs on apical third of lateral margin. Accessory seta (*7*) small, simple; terminal seta (*8*) short, three- to four-forked near middle.

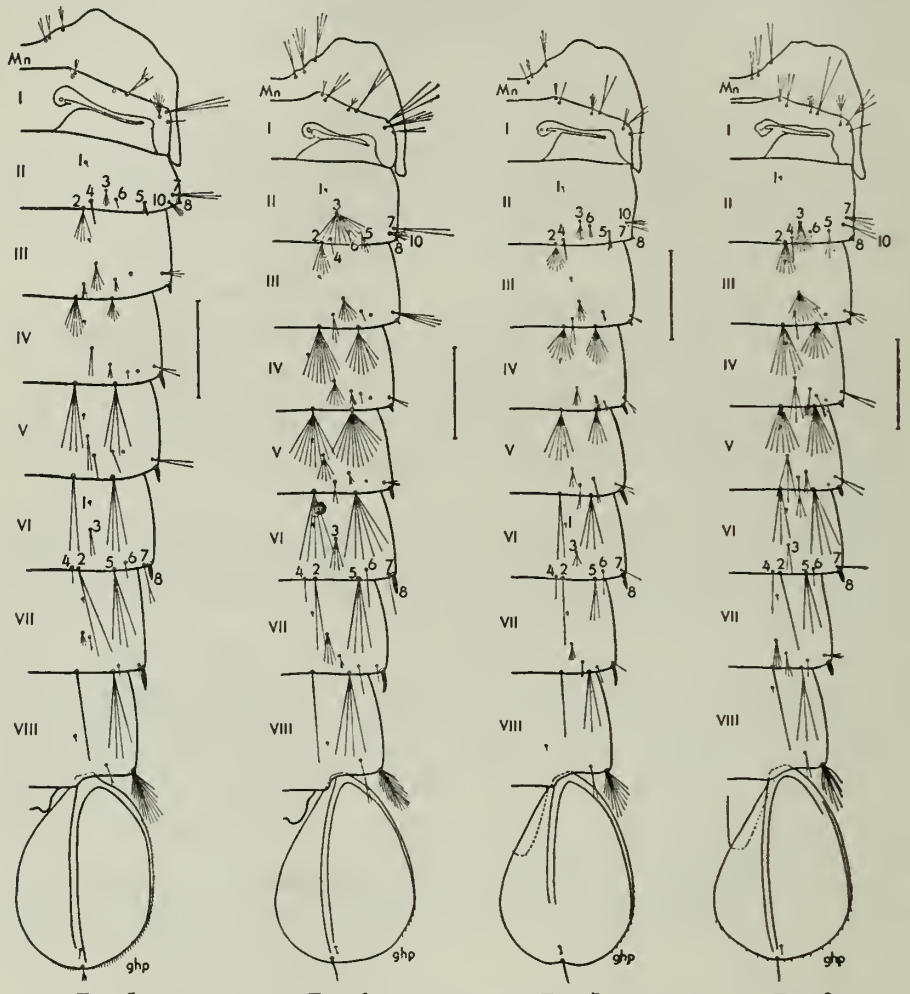


FIG. 5 FIG. 6 FIG. 7 FIG. 8

FIG. 5. *Anopheles atropos*. Right dorsal aspect of metanotum and abdomen of female from Florida.

FIG. 6. *Anopheles aztecus*. Right dorsal aspect of metanotum and abdomen of female from Mexico.

FIG. 7. *Anopheles punctipennis*. Right dorsal aspect of metanotum and abdomen of male from New York.

FIG. 8. *Anopheles earlei*. Right dorsal aspect of metanotum and abdomen of male from Michigan.

### ANOPHELES (ANOPHELES) AZTECUS Hoffmann (1935)

Pupa undescribed previously although Vargas (1943) gave a figure of the metanotum and abdominal segments, and Vargas and Matheson (1948) included it in a

key to pupae of the Nearctic *maculipennis* complex and included a photograph of the trumpets of specimens from Xochimilco, D.F., Mexico.

I am unaware of any records of this species north of the Mexican border, but since its affinities properly lie with the American species it has been included here.

*Specimens Examined.* Two pupal exuviae as follows: MEXICO: 2 ♀♀ from "Sta. Ursula Coapa," Distrito Federal, A. M. Palacios (*TU 138*).

*Cephalothorax.* Specimens littered with debris so that postocular, anterothoracic and supra-alar setae were indistinguishable. Dorsal seta (*8*) medium, simple. Metanotal setae: *10* medium, two- to four-forked at tip; *11* medium, four- to three-forked; *12* long, three-forked at tip.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* small, two- to five-forked; *4* medium, three- to six-forked; *5* long, three- to four-forked near tip; *6* small, three- to six-forked; *7* long, three- to two-forked; *8* medium, two-forked or simple; *10* long, four- to five-forked.

*Segment II: 1* very small, simple on this and all following segments; *2* medium, six-forked; *3* medium-long, nine- to eleven-forked; *4* small, simple or two-forked at tip; *5* small, four-forked; *6* small, two-forked; *7* long, two- or three-forked; *8* small, stout, transparent spine; *10* small, six- or seven-forked.

*Segment III:* Setae *3*, *4* and *6* rather unimportant on this and segments IV through VII; *2* medium, six- to ten-forked; *5* small, eight- to ten-forked; *7* medium-long, four-forked; *8* similar to and nearly twice the length of *8*-II.

*Segment IV: 2* long, nine- or ten-forked; *5* long, eight- to twelve-forked; *7* medium, two-forked; *8* similar to and about a third longer than *8*-III.

*Segment V: 2* long, five- or six-forked; *5* long, six- to four-forked; *7* small, three-forked; *8* darker, but nonpigmented, about twice the length of *8*-IV.

*Segment VI: 2* long, two-forked or simple; *5* long, four- to five-forked; *7* medium-long, simple; *8* similar to and slightly longer than *8*-V.

*Segment VII: 2* long, simple; *5* long, four-forked; *7* small, simple; *8* similar to and about same length as *8*-VI.

*Segment VIII: 5* medium-long, two- to three-forked near tip; *8* base similar to *8*-VI in shape and length, but with about sixteen smaller side branches.

*Paddle:* Margin entire, or with only three or four small spines near apex of buttress on lateral side. Accessory seta (*7*) small, simple or two-forked at tip; terminal seta (*8*) medium, stout, simple, straight.

### ANOPHELES (ANOPHELES) PUNCTIPENNIS (Say, 1823)

Pupa variously treated by a number of authors, including the following: Howard, Dyar and Knab (1912–1917) gave a figure of the whole pupa in lateral view and brief non-diagnostic notes; Senevet (1930) gave a complete detailed description and figure of the metanotum and dorsal abdominal segments of specimens from Illinois; Matheson (1944) gave a figure of the whole pupa in lateral view without descriptive notes; Aitken (1945) gave an incomplete description and figure of the terminal abdominal segments of specimens from California; Burgess (1946) described the dorsal pigmentation of living pupae and included a figure; and, Sabrosky (1946) included the species in a key to the pupae of Michigan anophelines.

*Specimens Examined.* Fourteen pupal exuviae as follows: CALIFORNIA: 1 ♀ from Green Valley, Solano County, VIII-29-46, RMB (*TU 704*). MEXICO: 2 ♂♂ and 1 ♀ from Ixmiquilpan, Hidalgo, J. Martinez (*TU 137*). NEW YORK: 1 ♀ from Cornell Fish Hatchery, Tompkins County, V-24-46, GHP, GHB (*TU 33*); 1 ♂ and 1 ♀ from 6 mi. e. Ithaca, Tompkins County, V-24-46, GHP, GHB (*TU 34*); 2 ♂♂ and 2 ♀ ♀ from Ithaca, Tompkins County, VI-25-47, RFD (*TU 133*); 2 ♂♂ and 2 ♀ ♀ from Ithaca, Tompkins County, VIII-5-47, RFD (*TU 134*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae all medium or small and otherwise inconspicuous. Metanotal setae as follows: *10* medium, usually simple, rarely two-forked near tip; *11* medium, usually three-forked, occasionally four- or five-forked; *12* long, usually three-forked, range from two- to four-forked.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* small, usually three-forked, occasionally four- or five-forked; *4* medium, simple or two-forked, rarely three-forked; *5* long, two- to three-forked; *6* medium, four- or five-forked, rarely two-forked; *7* medium, two- to three-forked at tip; *8* small, simple or rarely two-forked; *10* medium, two- to five-forked.

*Segment II: 1* small, simple on this and all following segments; *2* medium, four- to twelve-forked; *3* medium, seven- or six-forked, rarely four-forked; *4* medium, stout, simple; *5* medium, three- to four-forked; *6* small, two- to four-forked; *7* medium, usually two-forked, occasionally three- or four-forked; *8* small, transparent spine; *10* medium, four- to three-forked.

*Segment III:* Setae *3*, *4* and *6* medium or small and unimportant on this and segments IV through VII; *2* medium, usually eight-forked, range from six- to ten-forked; *5* medium, usually eight-forked, range from six- to nine-forked; *7* small, usually two-forked, rarely three-forked; *8* small, dark-pigmented, stout, a little more than twice the length of *8*-II.

*Segment IV: 2* long, six- to five-forked; *5* medium, five- to seven-forked; *7* medium, simple to two-forked; *8* similar to and a little less than twice the length of *8*-III.

*Segment V: 2* long, two- to three-forked; *5* long, four- to three-forked; *7* medium, simple or rarely two-forked near tip; *8* similar to and about a third longer than *8*-IV.

*Segment VI: 2* long, simple; *5* long, four- to three-forked; *7* medium, simple; *8* similar to and averaging slightly longer than *8*-V.

*Segment VII: 2* long, simple or occasionally two-forked; *5* long, three- or four-forked, or rarely six-forked; *7* small, simple; *8* similar to and averaging nearly twice the length of *8*-IV.

*Segment VIII: 5* medium, simple to two- or three-forked; *8* stout dark base with eight to eleven smaller side branches.

*Paddle:* Margin very finely serrate laterally at tip of buttress, either entire or with very fine, widely scattered hairs from there on around apex of paddle. Accessory seta (*7*) small, two-forked to simple; terminal seta (*8*) light brown, medium thin, simple, straight or slightly curved or hooked.

### ANOPHELES (ANOPHELES) EARLEI Vargas (1935)

Previously undescribed although Vargas (1943) included a few remarks and gave figures of the metanotum and abdomen of specimens from Cayuta Lake, N. Y.;

Matheson (1944) included a figure of the terminal abdominal segments under the name of *occidentalis*; and Vargas and Matheson (1948) included it in a key to Nearctic pupae of the *maculipennis* complex and gave figures of the metanotum, abdomen and trumpet of specimens from Cayuta Lake, N. Y.

*Anopheles earlei* has not been generally recognized as distinct from *occidentalis* of the west coast, although the characters shown recently by Vargas (1943) and by Vargas and Matheson (1948) in their illustrations of the pupa, and fully described here certainly point to the validity of *earlei* as a distinct species. All records of *occidentalis* from localities at least as far west as Wisconsin are probably referable to *earlei*.

*Specimens Examined.* Two pupal exuviae as follows: MICHIGAN: 1 ♂ from Bessy Creek at Douglas Lake, Cheboygan County, V-10-22, RM (*CU 1027–1712*). NEW YORK: 1 ♂ from Cayuta Lake, Schuyler County, V-30-42, RM (*CU 1027–1333*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae all medium or small and unimportant except seta *7* which is long and simple. Metanotal setae as follows: *10* medium, two-forked near tip; *11* medium, two-forked; *12* long, two-forked or simple.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* medium, two- to four-forked; *4* medium, two- to four-forked; *5* long, two-forked; *6* small, five- to three-forked; *7* long, two- or three-forked; *8* small, simple or two-forked; *10* medium, two- to four-forked.

*Segment II: 1* very small, simple on this and all following segments; *2* medium, six-forked; *3* medium, four- to eight-forked; *4* medium, simple; *5* medium, three- or five-forked; *6* small, three- or two-forked; *7* medium, three- or two-forked; *8* small, stout, transparent spine; *10* medium, two- or three-forked.

*Segment III:* Setae *3*, *4* and *6* rather unimportant on this and segments IV through VII; *2* long, six- or seven-forked; *5* medium, eight- to ten-forked; *7* medium, three- or two-forked; *8* similar to and ranging from equal to twice the length of *8*-II.

*Segment IV: 2* long, five- to seven-forked; *5* long, six- to ten-forked; *7* medium, two-forked to simple; *8* non-pigmented, though darker, and ranging from one-third longer to twice the length of *8*-III.

*Segment V: 2* long, three- to two-forked; *5* long, three- to five-forked; *7* medium, simple or two-forked; *8* similar to and slightly longer than *8*-IV.

*Segment VI: 2* long, simple or two-forked; *5* long, three- to five-forked; *7* medium, simple; *8* similar to and slightly longer than *8*-V.

*Segment VII: 2* long, simple; *5* long, two- to five-forked; *7* small, three- or four-forked; *8* similar to and slightly longer than *8*-VI.

*Segment VIII: 5* medium, two-forked near tip; *8* transparent, base about equal to *8*-VII, with eight to twelve smaller side branches.

*Paddle:* Apical third of lateral and medial margins with a fringe of fine short hairs. Accessory seta (*7*) small, simple; terminal seta (*8*) small, straight, simple.

### ANOPHELES (ANOPHELES) GEORGIANUS King (1939)

Pupa heretofore undescribed although Burgess (1946) presented a few notes on the pigmentation of living pupae.

*Specimens Examined.* Five pupal exuviae as follows: GEORGIA: 1 ♂ and 4 ♀ ♀ from Quitman, Brooks County, II-16-38, WVK No. 1957 (*USNM*).

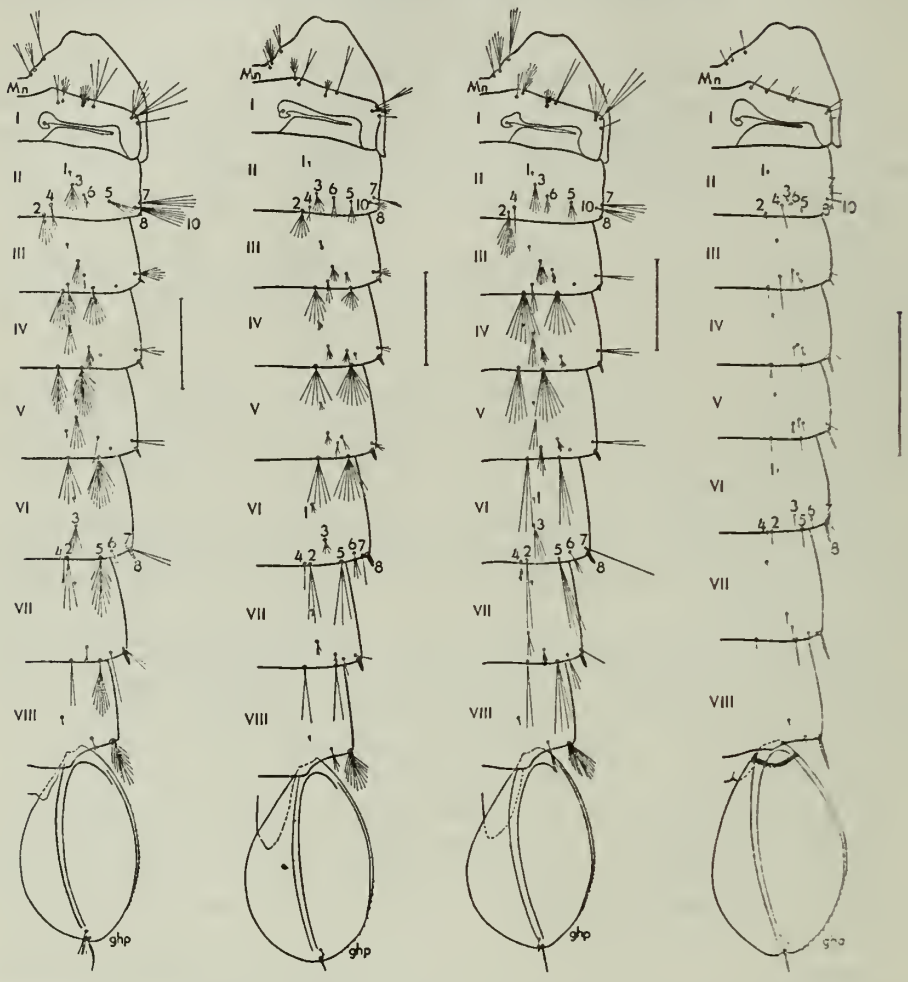


FIG. 9 FIG. 10 FIG. 11 FIG. 12

FIG. 9. *Anopheles georgianus.* Right dorsal aspect of metanotum and abdomen of female from Georgia.

FIG. 10. *Anopheles crucians.* Right dorsal aspect of metanotum and abdomen of male from Louisiana.

FIG. 11. *Anopheles bradleyi.* Right dorsal aspect of metanotum and abdomen of male from Florida.

FIG. 12. *Anopheles barberi.* Right dorsal aspect of metanotum and abdomen of female from Missouri.

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae all medium in size and unimportant except seta *7* which is long and simple or two-forked. Metanotal setae as follows: *10* medium, simple or two- to three-forked near tip; *11* medium, four- to seven-forked; *12* medium-long, three- or four-forked.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* small, two- or three-forked; *4* small, five- to eight-forked; *5* long, three- or four-forked, rarely two-forked; *6* small, seven- to nine-forked; *7* long, three- to six-forked; *8* medium, simple, or rarely two-forked near tip; *10* medium, five- to nine-forked.

*Segment II: 1* small, simple on this and all following segments; *2* medium, five- to seven-forked near base; *3* medium, four- to seven-forked; *4* medium, rather stout, three- to seven-forked near apex; *5* medium, four- to six-forked; *6* small, six- to seven-forked; *7* long, usually four-forked, rarely three-forked; *8* small, thin, transparent spine; *10* long, four- to seven-forked.

*Segment III:* Setae *3*, *4* and *6* rather inconspicuous, small medium and rather unimportant on this and segments IV through VII; *2* medium-long, seven- to ten-forked; *5* medium, six- to twelve-forked; *7* medium, four- to six-forked; *8* small, dark-pigmented spine, roughly twice the length and diameter of *8*-II.

*Segment IV: 2* long, eight- to sixteen-branched; *5* long, thirteen- to twenty-branched; *7* medium, usually three-forked, rarely two-forked; *8* stout, dark spine averaging twice the length and diameter of *8*-III.

*Segment V: 2* long, five- to seven-forked; *5* long, nine- to fourteen-forked; *7* medium, usually two-forked, rarely simple or three-forked; *8* similar to and averaging slightly longer than *8*-IV.

*Segment VI: 2* long, two- to six-forked; *5* long, eight- to eleven-forked; *7* medium two-forked; *8* similar to and averaging slightly longer than *8*-V.

*Segment VII: 2* long, two- to three-forked, rarely simple; *5* long, seven- to eleven-forked; *7* small, two-forked, rarely three-forked; *8* similar to and averaging slightly longer than *8*-VI.

*Segment VIII: 5* small, two- to four-forked; *8* with a stout dark base similar to *8*-VII and ten to eighteen smaller side branches.

*Paddle:* Lateral margin with short, very fine teeth from the base three-fourths of the way to the apex. Accessory seta (*7*) small, two- to four-forked near basal third; terminal seta (*8*) medium, dark-pigmented, slightly curved.

### ANOPHELES (ANOPHELES) CRUCIANS Wiedemann (1828)

Pupa heretofore undescribed although Mitchell (1907) included figures of the whole pupa in lateral aspect and a ventral view of the paddles, and Burgess (1946) gave notes on the dorsal pigmentation of the living pupae.

*Specimens Examined.* Seven pupal exuviae as follows: LOUISIANA: 1 ♂ and 1 ♀ from Waggaman, Jefferson Parish, X-17-47, LLE, JVB (*TU 116*); 3 ♀♀ from 3 mi. sw. Franklinton, Washington Parish, VIII-13-48, GHP, MHP (*TU 779*); 1 ♀ from Covington, St. Tammany Parish, VIII-16-48, GHP, MHP (*TU 796*); 1 ♀ from 3 mi. n. Franklin, St. Mary Parish, VIII-25-48, GHP, MHP (*TU 105*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae small or medium and rather inconspicuous except seta *7* which is long, thin and simple. Metanotal setae as follows: *10* medium, simple; *11* medium, five- or six-forked, or rarely three-forked; *12* medium, usually three-forked, range from two- to five-forked.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* medium, six- to eight-forked; *4* long, usually two-forked, occasionally simple; *5* long, two- or three-forked

near apex; *6* medium-small, four- to eleven-forked; *7* long, usually three-forked near middle, range from simple to four-forked; *8* small, simple or rarely two-forked; *10* small, usually five-forked, range from three- to seven-forked.

*Segment II: 1* small, simple on this and all following segments; *2* small, seven- to six-forked; *3* small, usually six-forked, occasionally eight-forked; *4* small, stout, two-forked at tip to simple; *5* small, three- to four-forked, rarely two-forked; *6* small, three-forked; *7* medium, two- or three-forked, or occasionally simple; *8* very small, transparent, thin spine; *10* small, two- to seven-forked.

*Segment III:* Setae *3, 4* and *6* all small and unimportant on this and segments IV through VII; *2* medium, seven- to twelve-forked; *5* medium, eight- to fourteen-forked; *7* small, two- to eight-forked; *8* dark-pigmented, stout, about twice the length of *8*-II.

*Segment IV: 2* medium, six- to ten-forked; *5* medium, nine- to eleven-forked; *7* small, three- or four-forked, rarely simple; *8* similar to and about twice the length of *8*-III.

*Segment V: 2* medium, three- to five-forked; *5* medium, eight- to four-forked; *7* small, three- or two-forked, rarely simple; *8* similar to and slightly longer than *8*-IV.

*Segment VI: 2* medium, three- or two-forked; *5* medium, three- to six-forked; *7* small, simple; *8* similar to and slightly longer than *8*-V.

*Segment VII: 2* medium, two-forked to simple, rarely three-forked; *5* medium, two- to five-forked, rarely simple; *7* small, simple; *8* similar to and about same length as *8*-VI.

*Segment VIII: 5* medium-small, two- or three-forked, or occasionally four-forked; *8* with base about equal to *8*-VII with twelve to fifteen smaller side branches.

*Paddle:* Margin with evenly spaced fine teeth on lateral side. Accessory seta (*7*) very small, simple or two-forked; terminal seta (*8*) small, stout, simple.

### ANOPHELES (ANOPHELES) BRADLEYI King (1939)

Pupa heretofore undescribed or figured.

*Specimens Examined.* Twenty pupal exuviae as follows: FLORIDA: 2 ♂♂ and 1 ♀ from Brevard County, II-25-38, T. E. McNeel, WVK No. 1958 (*USNM*). LOUISIANA: 4 ♂♂ and 3 ♀ ♀ from Michaud, Orleans Parish, I-9-48, GHP, LLE, JVB (*TU 140*). SOUTH CAROLINA: 1 ♂ and 1 ♀ from Parris Island, Beaufort County, VII-28-45, GHB (*TU P-515*); 1 ♂ and 5 ♀ ♀ from Horse Island, Beaufort County, X-18-45, GHB (*TU P-527*); 1 ♂ and 1 ♀ from Ladies Island, Beaufort County, X-21-45, GHB (*TU P-528*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae all medium in size and rather unimportant except seta *7* which is long and simple. Metanotal setae as follows: *10* long, two-forked above middle, occasionally three- or four-forked above middle; *11* long, four- or five-forked, rarely two- to three-forked; *12* long, three- to four-forked.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* medium, two-forked, occasionally three- or four-forked; *4* medium, various, range from two- to nine-forked; *5* long, two- to three-forked; *6* small, usually six- or seven-forked, range from

two- to eight-forked; *7* long, two- to four-forked; *8* medium, simple to two-forked; *10* medium, four- to six-forked.

*Segment II: 1* small, simple on this and all following segments; *2* long, five- to eleven-forked; *3* medium, five- to nine-forked; *4* medium, stout, simple, or rarely two-forked near tip; *5* small, three- or four-forked; *6* small, three- to seven-forked; *7* long, two- to four-forked; *8* small, thin, transparent spine; *10* two- to four-forked, slightly shorter than *7*.

*Segment III:* Setae *3*, *4* and *6* small to medium in size, for the most part inconspicuous and unimportant on this and segments IV through VII; *2* medium-long, six- to nine-forked; *5* medium, four- to seven-forked; *7* medium, two- to four-forked; *8* small, dark, stout, about twice the length and diameter of *8*-II.

*Segment IV: 2* long, six- to nine-forked; *5* long, seven- to ten-forked; *7* medium, nearly three times the length of *8*, three-forked; *8* similar to and about twice the length and diameter of *8*-III.

*Segment V: 2* long, two- to six-forked; *5* long, four- to eight-forked; *7* long, two-forked; *8* similar to and averaging slightly longer than *8*-IV.

*Segment VI: 2* long, two- to four-forked, or occasionally simple; *5* long, three- to six-forked; *7* long, simple, or rarely two-forked at tip; *8* similar to and averaging slightly longer than *8*-V.

*Segment VII: 2* long, two-forked, or rarely simple; *5* long, three- to five-forked; *7* small, roughly twice the length of *8*, two-forked, or rarely simple; *8* similar to and averaging slightly longer than *8*-VI.

*Segment VIII: 5* medium, two- to four-forked; *8* short, dark basal spine with nine to sixteen smaller side branches.

*Paddle:* Basal four-fifths of lateral margin with evenly spaced short fine teeth; apical fifth entire. Accessory seta (*7*) very small, usually three-forked, occasionally simple or two-forked; terminal seta (*8*) small, dark, simple, usually straight.

### ANOPHELES (ANOPHELES) BARBERI Coquillett (1903)

Pupa heretofore undescribed.

*Specimens Examined.* Five pupal exuviae as follows: ALABAMA: 1 ♂ and 2 ♀ ♀ from Florence, Lauderdale County, IX-14-48, W. E. Snow (*TU 800*). MISSOURI: 2 ♀ ♀ from Camp Crowder, Newton County, VII-20-42 and IX-16-42, A. B. Gurney (*USNM*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae all small or medium and rather inconspicuous except seta *7* which is long and three-forked. Metanotal setae as follows: *10* small, simple or two-forked; *11* small, simple; *12* medium, simple or two-forked.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* small, simple; *4* small, simple to five-forked; *5* small, simple or two-forked; *6* small, simple to four-forked; *7* small, simple to three-forked; *8* small, simple; *10* small, simple to two-forked.

*Segment II: 1* very small, simple on this and all following segments; *2* small, simple to three-forked; *3* small, simple; *4* medium, simple to three-forked near tip; *5* small, four- to eight-forked; *6* small, simple; *7* small, simple to two-forked; *8* small, thin transparent seta; *10* small, two-forked.

*Segment III:* Setae *3*, *4* and *6* all small and inconspicuous on this and segments IV through VII; *2* small, simple; *5*, small, simple; *7* small, simple to three-forked; *8* thin, dark-pigmented, about three times the length of *8*-II.

*Segment IV: 2* small, two-forked; *5* small, simple; *7* small, simple or three-forked; *8* stouter than and about a third longer than *8*-III.

*Segment V: 2* small, simple to three-forked; *5* small, simple; *7* small, simple to three-forked; *8* similar to and slightly longer than *8*-IV.

*Segment VI: 2* small, simple; *5* medium, stout, simple; *7* small, two- to four-forked near tip; *8* similar to and about the same length as *8*-V.

*Segment VII: 2* small, simple or two-forked; *5* medium-long, stout, simple; *7* small, simple; *8* similar to and nearly twice the length of *8*-VI.

*Segment VIII: 5* small, simple; *8* similar to and a little more than twice the length of *8*-VI, without side branches.

*Paddle:* With a series of widely spaced, fine, sharp teeth on apical half of lateral margin. Accessory seta (*7*) very small, simple or two-forked; terminal seta (*8*) medium, stout, simple, straight.

### ANOPHELES (ANOPHELES) FRANCISCANUS McCracken (1904)

Following the conclusions of Aitken (1945) I have considered all western United States specimens of the *pseudopunctipennis* complex as being the form described as *franciscanus* by McCracken. The pupa of this species was partially described and illustrated by Aitken (1945) from California. Typical Mexican *pseudopunctipennis* may occur in Texas and western Louisiana, but no pupal specimens from this area were available in connection with this study. However, a single pupal exuviae in the USNM from Ancon, Canal Zone which I examined did not show characters which would distinguish it from Californian or New Mexican specimens of *franciscanus*. Senevet (1934) described the pupa of *pseudopunctipennis* but his specimens were also from the Canal Zone.

*Specimens Examined.* Fourteen pupal exuviae as follows: CALIFORNIA: 2 ♀♀ from Van Nuys, Los Angeles County, W. W. Farrar (*USNM*); 2 ♂♂ and 1 ♀ from Monticello, Napa County, X-8-47, RMB (*TU 708*). NEW MEXICO: 3 ♀♀ from Isleta, Bernalillo County, VI-21-48, A. Miller, J. Gonzales (*TU 441*); 2 ♂♂ and 4 ♀♀ from Las Cruces, Dona Ana County, VI-24-48, A. Miller, J. Gonzales (*TU 442*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae all small or medium or otherwise inconspicuous except seta *7* which is very long and simple. Metanotal setae as follows: *10* medium, simple or two-forked; *11* small, simple to three-forked near tip; *12* medium, two-forked at tip.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* medium, simple to three-forked; *4* medium, simple to two-forked; *5* long, three- or two-forked; *6* small, four-forked, range from simple to five-forked; *7* long, simple; *8* medium, two-forked, range from simple to three-forked near tip; *10* medium, four-forked, range from three- to five-forked.

*Segment II: 1* very small, simple on this and all following segments; *2* medium-long, six- to three-forked; *3* medium, four-forked, range from three- to five-forked; *4* medium, simple or two-forked near tip; *5* small, four-forked, range from three- to

five-forked; *6* small, two- to five-forked; *7* long, simple or two-forked at tip; *8* a thin, transparent seta nearly twice the length of *8*-III; *10* medium, three-forked, or four- to five-forked.

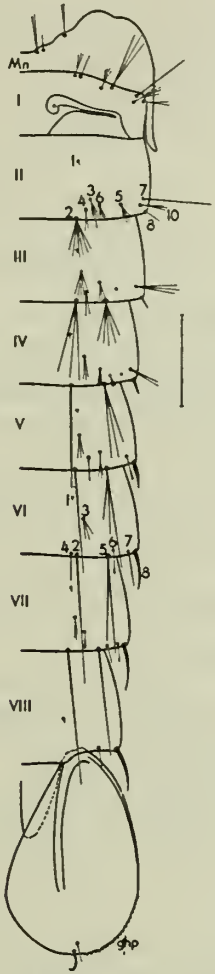


Fig. 13

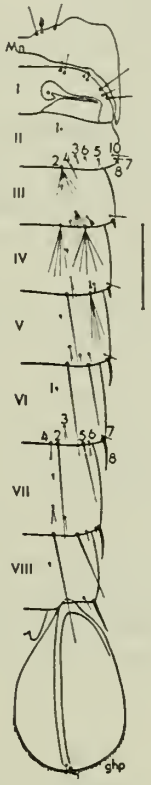


Fig. 14

Fig. 13. *Anopheles franciscanus.* Right dorsal aspect of metanotum and abdomen of male from New Mexico.

Fig. 14. *Anopheles albimanus.* Right dorsal aspect of metanotum and abdomen of female from Texas.

*Segment III:* Setae *3*, *4* and *6* small or unimportant on this and segments IV through VII; *2* very long, three-forked; *5* medium-long, five-forked, range from four- to nine-forked; *7* medium, three- or four-forked; *8* short, stout, nonpigmented.

*Segment IV: 2* very long, two-forked, range from simple to three-forked; *5* very long, three-forked; *7* medium, two- to four-forked; *8* similar to and averaging a little less than twice the length of *8*-III.

*Segment V: 2* very long, simple to two-forked; *5* very long, two- to three-forked; *7* medium, two- or three-forked; *8* similar to and averaging about twice the length of *8*-IV.

*Segment VI: 2* very long, simple or two-forked; *5* very long, two-forked; *7* medium, two-forked at tip or simple; *8* similar to and averaging about a third longer than *8*-V.

*Segment VII: 2* very long, simple; *5* very long, two-forked or simple; *7* small, simple or two-forked at tip; *8* similar to and averaging slightly longer than *8*-VI.

*Segment VIII: 5* small, simple or two-forked; *8* similar to 8-VII, but slightly shorter, without side branches.

*Paddle:* Postero-lateral margin with a row of rather stout, slightly hooked short spines which reach to the apex of the paddle. Accessory seta (*7*) small, simple or two-forked near tip; terminal seta (*8*) medium, definitely curved and hook-like.

### ANOPHELES (NYSSORHYNCHUS) ALBIMANUS Wiedemann (1821)

The pupa was included in a key to seven Neotropical species of the subgenus *Nyssorhynchus* by Root (1926), and Senevet (1931) gave a complete description and figures of the trumpet, metanotum and abdomen of specimens without specific locality data.

*Specimens Examined.* Six pupal exuviae as follows: MEXICO: 1 ♀ from Villahermosa, Tabasco (*TU 177*). TEXAS: 2 ♂♂ and 3 ♀♀ from Brownsville, Cameron County, IX-17-44, T. M. Burns (*USNM*).

*Cephalothorax.* Postocular, anterothoracic, dorsal and supra-alar setae all rather small and inconspicuous. Metanotal setae as follows: *10* and *12* simple, subequal in length; *11* small, simple to three- or four-forked.

*Abdomen.* All dorsal setae non-plumose. *Segment I: 3* small, two- or three-forked; *4* small, simple; *5* small, simple to three-forked; *6* small, simple or two-forked; *7* small, simple; *8* medium, simple; *10* long, simple.

*Segment II: 1* small, simple on this and all following segments; *2* medium-long, six- or seven-forked, range from three- to eight-forked; *3* small, four- to six-forked; *4* medium, stout, simple; *5* small, two- or three-forked; *6* small, simple; *7* small, simple or two-forked; *8* small, stout, transparent spine with blunt tip; *10* medium, simple.

*Segment III:* Setae *3*, *4* and *6* all small and inconspicuous on this and segments IV through VII; *2* long, usually four-forked, range from three to six; *5* long, usually seven-forked, range from six- to nine-forked; *7* medium, simple or rarely two-forked near tip; *8* dark brown pigmented, stout, about twice as long as *8*-II.

*Segment IV: 2* long, simple; *5* usually four-forked, occasionally five- to seven-forked; *7* small, simple; *8* similar to and slightly longer than *8*-III.

*Segment V: 2* long, simple; *5* long, simple; *7* small, simple; *8* dark, brown-pigmented, curved medially and tapered sharply to apex, about three times the length of *8*-IV.

*Segment VI: 2* long, simple; *5* long, simple; *7* small, simple; *8* similar to and slightly longer than *8*-V.

*Segment VII: 2* long, simple; *5* long, simple; *7* very small, simple; *8* similar to and about same length as *8*-VI.

*Segment VIII:* 5 small, simple or two-forked; 8 similar to and about same length as 8-VII.

*Paddle:* Apical third of paddle with a short fringe of fine hairs extending around the apex. Accessory seta (7) short, simple; terminal seta (8) stout, simple, and slightly curved.

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# ADRENAL HYPERTROPHY IN CHICKS INFECTED WITH *PLASMODIUM GALLINACEUM*

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A number of investigators have reported pathological changes in adrenal glands associated with plasmodial infections (Paisseau and Lamaire, 1916; Natali, 1934; Spitz, 1946; Golden and Overman, 1948). Other investigators have described adrenal insufficiency in human malaria patients (Fraga, 1917; Junior and Brandão, 1937; Chessa, 1938; Marañon, 1939). No descriptions of adrenal hypertrophy associated with malaria have been found in the literature.

While studying the effect of infections with the 8A strain of *Plasmodium gallinaceum* Brumpt on the ascorbic acid content of chick adrenals (Josephson, *et al.*, 1949), we observed that adrenal hypertrophy was usually associated with heavy infections. Observations were extended to attempt to correlate the adrenal hypertrophy with the course of this malarial infection, which is predictable within narrow ranges and the intensity of which can be readily followed by frequent estimation of parasitemia.

## METHODS

The chicks used throughout the course of this work were New Hampshire Reds which, at the time of infection, were one week old and weighed between 42 and 55 grams. Experimental and control groups were kept in brooders under identical conditions. Unless otherwise indicated, they were allowed access to water and starting mash *ad libitum*. Red blood cell counts of all chicks were made each morning. Smears were made from the infected chicks at the same time and examined microscopically for parasitemia. During various stages of the infection the chicks were sacrificed individually by either cervical dislocation or chloroform. This was done 2 to 3 hours following the morning reading of the red blood cell and parasite counts. The adrenals were removed by careful dissection immediately after death.

The experimental group of chicks was inoculated intravenously with blood containing 1,000 parasitized erythrocytes obtained from chicks infected with *P. gallinaceum*. With this inoculum the course of parasitemia is generally as shown in figure 1. Initial parasitemia is detectable microscopically in a blood film on day 5 or 6 after inoculation. More than one-half the chicks die while the numbers of parasites in the blood are increasing. A peak parasitemia (where 80 to 90 per cent of the erythrocytes are invaded) is reached in the surviving chicks on day 10 or 11 after inoculation. The course of infection following peak parasitemia is character-

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ized by a drop in parasite density and the appearance of large numbers of reticulocytes (50 to 75 per cent of all erythrocytes). In some chicks the decrease in parasitemia is dramatic, densities falling to levels almost undetectable in routine examination of blood smears. In most chicks, however, where the reduction in parasitemia is moderate, about 40 to 60 per cent of the total number of erythrocytes and reticulocytes in circulation are parasitized. The survivors of peak parasitemia ultimately die between 15 and 25 days after inoculation as a result of a proliferation of exoerythrocytic parasites in the endothelial cells of vital organs. During the course of the

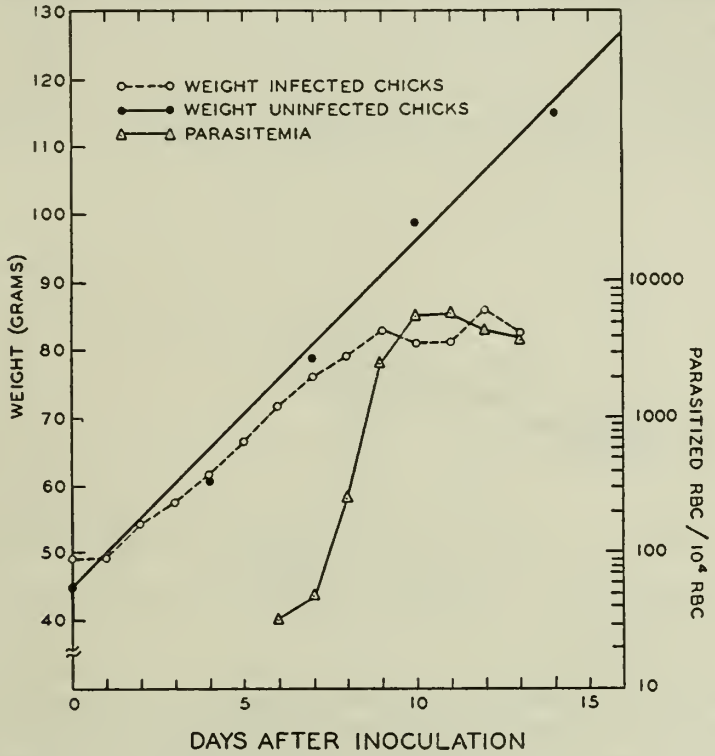


FIG. 1. Mean course of parasitemia and increase in weight in 10 infected chicks. For comparative purposes, the mean weight increase for 10 uninfected chicks of the same lot is shown.

infection the birds become lethargic; there is an apparent loss of appetite which is reflected in the smaller weight gains achieved by infected chicks compared with the weight gains in uninfected controls (figure 1).

Immediately after the chicks are sacrificed, an incision is made through the skin along the junction of the body wall and left femur. The left thigh is hyperextended, dislocated and the incision carried down through the peritoneum.

The intestinal loops, liver, spleen, etc., are rotated to the right side, exposing the organs that lie against the vertebral column. A block dissection, approximately 2 cm. square, is made of the entire area under investigation. This block includes vertebral column, gonads, adrenals, connective tissue, blood vessels, and posterior

portions of the lungs and the anterior poles of the kidneys. A careful dissection is then made from the block. Sections of various organs are taken as needed and placed in 10 per cent formalin for histopathological study.

In female chicks, care must be taken to separate the ovarian tissue from the adrenals which lie partially embedded in the broad anterior base of the large lobulated single ovary. This is done most easily if the dissecting scissors snip close to the vertebral column, while the forceps, holding the pointed posterior ovarian pole, draws the ovarian tissue off the vertebral column. A low power dissecting lens helps identify the ovoid orange-yellow adrenal bodies from the yellow-white lobulated ovarian tissue. As parasitemia develops, the adrenals may assume a brown-stained appearance. In male chicks, the dissection is relatively simple.

The two adrenals are removed together and later separated by cutting along the path of the blood vessels coursing between them. Each adrenal gland is then dissected clear, and placed in a petri dish surrounded by cracked dry ice, and either

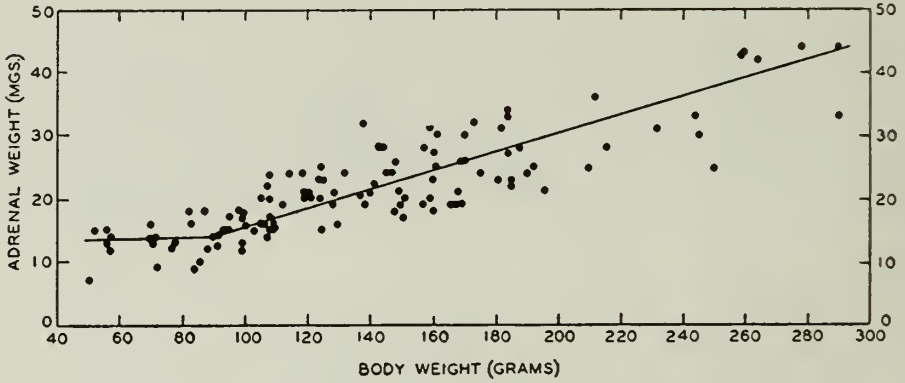


FIG. 2. Relationship between fresh adrenal weight and body weight of uninfected chicks

weighed immediately or when 3 to 5 dissections are completed. For moisture determinations, 3 pairs of fresh adrenal glands are placed in a weighing bottle and the glands are dried to a constant weight over $CaCl_2$ *in vacuo* at room temperature. When special staining procedures require unfixed fresh tissue, the organ sections are processed immediately after dissection. Chemical and histological changes are reduced to a minimum by completing each dissection within 10 minutes of the time of sacrificing.

## RESULTS

The weights in milligrams of pairs of fresh adrenals (AD) from 62 uninfected female chicks and 54 uninfected males as a function of total body weights in grams (W) of the same chicks are shown in figure 2. For $W \geqq 90$ or when the age of the chicks $\geqq$ 14 days, AD increases directly with age and W. AD/W in 106 chicks of age $\geqq$ 14 days is constant and equal to $0.156 \pm 0.0034$ (normal value line in figure 3).

In three experiments, 75 chicks were sacrificed during the period when parasitemia was still increasing. Fifty-seven additional chicks were sacrificed at various periods

after peak parasitemia. The AD/W of each chick is shown plotted against the number of infected erythrocytes at the time of sacrifice (figure 3). In 53 chicks with less than 50 per cent of the erythrocytes parasitized, AD/W is 0.178 ± 0.0046. However, compared with the ratio derived above for uninfected controls (0.156 ± 0.0034) there is a small but significant difference ($P<0.01$). This difference probably indicates that there has been a slight change in AD/W from the day of inoculation to the time of 50 per cent parasitemia. The AD/W rises sharply when over 50 per cent of the erythrocytes are parasitized and reaches a maximum folllowing

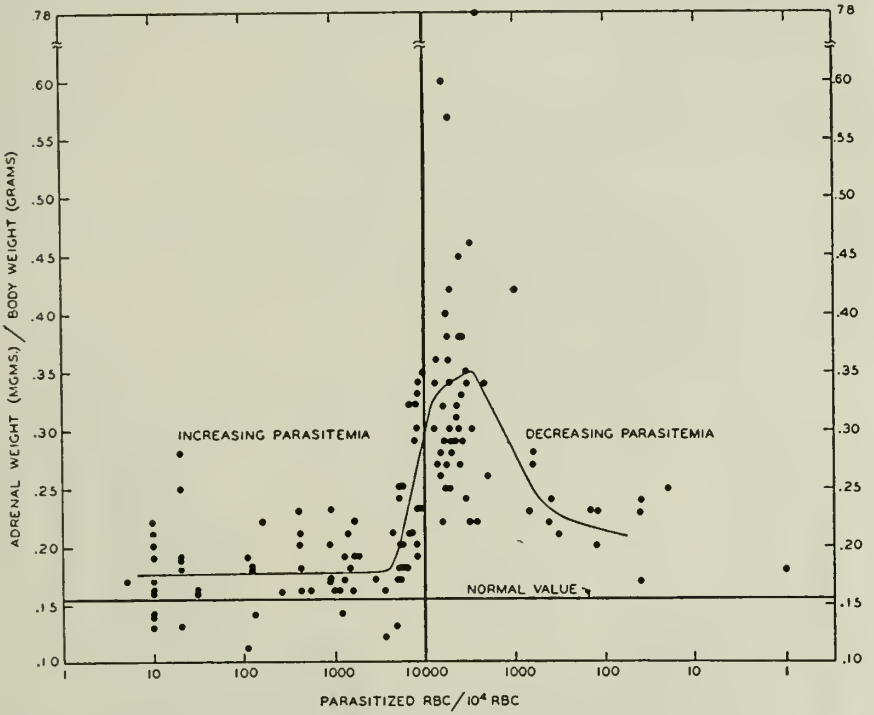


FIG. 3. Relationship between AD/W and degree of parasitemia. The AD/W for uninfected chicks is indicated by the horizontal line labelled "normal value."

peak parasitemia when the per cent of infected erythrocytes has declined to about 30. At the maximum, AD/W averages about 100 per cent higher than in corresponding uninfected chicks, and in one chick it is over 500 per cent higher.[2] There is an increase in the variability of AD/W among chicks sacrificed after peak parasitemia. In those chicks in which the per cent of infected erythrocytes declines further, AD/W approaches normal. With these last chicks there is no evidence that they ever responded to the infection with marked adrenal hypertrophy.

The greater AD/W values of infected chicks cannot be explained by their failure to gain weight. In table 1, body and adrenal weight gains of infected chicks are

[2] Since adrenal weight is a function of both body weight and age, the data were also analyzed to correct for both factors, and yielded essentially the same results.

TABLE 1

*Comparison of body and adrenal weight gains of chicks inoculated with* Plasmodium gallinaceum *with those of uninfected controls*

Data in last 6 columns are mean values

| GROUP | DAYS AFTER INOCULATION | NO. CHICKS | CLASS | % ERYTHROCYTES PARASITIZED | BODY WT. (W) (gm.) | ADRENAL WT. (AD) (mg.) | BODY WT. GAIN (gm.) | ADRENAL WT. GAIN (mg.) | AD/W |
|---|---|---|---|---|---|---|---|---|---|
| A | 6 | 9 | Control | — | 79.3 | 11.8* | — | — | 0.15 |
| B | 6 | 9 | Infected | 0.2 | 77.9 | 14.0 | — | — | 0.18 |
| C | 14 | 10 | Control | — | 137.5 | 18.6† | 58.2 | 6.8 | 0.14 |
| D | 14 | 10 | Infected | 39 | 97.1 | 29.2 | 19.2 | 15.2 | 0.30 |
| E | 15 | 10 | Control | — | 148.0 | 23.1‡ | 68.7 | 11.3 | 0.16 |
| F | 15 | 12 | Infected | 35 | 83.0 | 33.8 | 5.1 | 19.8 | 0.41 |
| G | 16 | 15 | Control | — | 143.6 | 21.5‖ | 64.3 | 9.7 | 0.15 |
| H | 16 | 20 | Infected | 24 | 118.9 | 29.0 | 41.0 | 15.0 | 0.24 |

* Group A vs. Group B, difference between the means ($\Delta$) = 2.2; $P > 0.2$.
† Group C vs. Group D, $\Delta = 10.6$; $P < 0.01$.
‡ Group E vs. Group F, $\Delta = 10.7$; $P < 0.01$.
‖ Group G vs. Group H, $\Delta = 7.5$; $P < 0.01$.

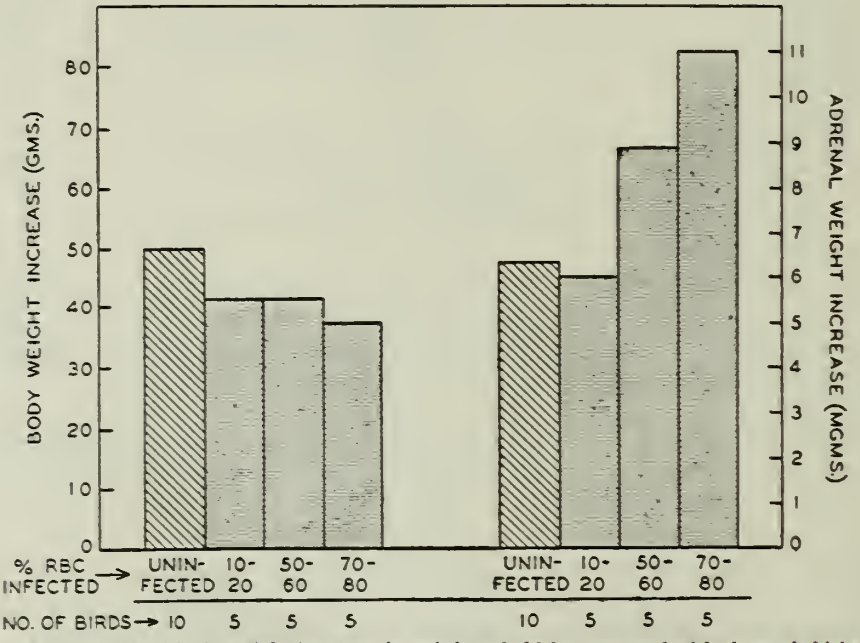


FIG. 4. Adrenal and body weight increases in uninfected chicks compared with those of chicks at different stages of parasitemia.

compared with those of normal chicks of the same age. When compared with the weights of chicks in Groups A and B, which were sacrificed eight days previously,

the control chicks in Group C had gained over three times as much in body weight as the infected chicks in Group D but less than half as much in adrenal weight. Both these facts are reflected in the AD/W of the infected chicks which is 0.30 compared with 0.14 for the controls. Similar results are observed for chicks in Groups E and F and Groups G and H. The results of another similar series are presented in figure 4.

TABLE 2

*Comparison of wet and dry adrenal weights of chicks infected with* Plasmodium gallinaceum *with those of uninfected controls*

| NO. CHICKS | MEAN % ERYTHROCYTES PARASITIZED | ADRENALS | | |
|---|---|---|---|---|
| | | Total Wet Wt. (mg.) | Total Dry Wt. (mg.) | % Moisture |
| 21-day-old, infected | | | | |
| 4 | 40 | 90.3 | 22.3 | 75.3 |
| 3 | 38 | 67.0 | 16.3 | 75.7 |
| 3 | 26 | 55.0 | 15.1 | 72.5 |
| 3 | 61 | 74.4 | 17.9 | 75.9 |
| 3 | 31 | 86.8 | 20.4 | 76.5 |
| 3 | 57 | 87.5 | 21.0 | 77.0 |
| 3 | 31 | 71.9 | 16.3 | 77.3 |
| Mean..... | 41 | 76.1 | 18.3 | 75.8 |
| 21-day-old, controls | | | | |
| 3 | — | 62.6 | 14.8 | 76.4 |
| 3 | — | 55.9 | 12.5 | 77.6 |
| 3 | — | 68.2 | 15.3 | 77.6 |
| 3 | — | 63.5 | 15.4 | 75.7 |
| 3 | — | 68.5 | 17.4 | 74.6 |
| 3 | — | 66.4 | 17.1 | 74.2 |
| 3 | — | 75.6 | 18.6 | 75.4 |
| Mean..... | — | 65.8 | 15.9 | 75.9 |

It was next of interest to determine whether the increase in adrenal weight of infected chicks was the result of an increase in tissue fluids or an actual increase in total tissue substance. For this purpose groups of 3 or 4 pairs of adrenals from infected and from normal chicks were weighed immediately after dissection and then dried to constant weight *in vacuo* at room temperature. The 21-day-old chicks (table 2) were sacrificed when peak parasitemia was passed. While the mean fresh and dry weights of the adrenals of the infected chicks is greater than the controls, it will be noted that there is no significant difference in mean per cent moisture content of the adrenals of the two groups. The tissue weight increments of the adrenals of the infected chicks contain the same proportions of mositure and solids as the normal adrenal.

It has been shown that in pigeons (McCarrison, 1919), dogs (Vincent and Hollenberg, 1928), and rats (Cameron and Carmichael, 1946), starvation will result in adrenal enlargement. Infected chicks with food *ad libitum* show marked retardation in weight gain and even lose weight (figure 1). This is partially attributable to loss of appetite. An inanition-control experiment was therefore conducted. Forty 18-day-old chicks were divided at random into 3 groups, X, Y, and Z. Group X (15 chicks) was sacrificed immediately to obtain base data for body weights and fresh adrenal weights. Group Y (10 chicks) was allowed access to standard ration and water *ad libitum*. Group Z (15 chicks) was given no food or water. At the end of 5 days, Groups Y and Z were sacrificed. It may be seen (table 3) that in those chicks allowed an adequate diet (Group Y) there was a parallel increase in body weights and adrenal weights (58 and 55 per cent of the base weight, respectively). The starved chicks (Group Z) had lost an average of 38 per cent of their original body weight, yet their adrenal weights were the same as those of the control birds killed initially (Group X). The adrenal tissue was apparently spared at the

TABLE 3

*Body weight (W) and adrenal weight (AD) changes in normal and starved chicks*

Data in last seven columns are mean values

| GROUP | DATE | NO. CHICKS | DIET PRIOR TO SACRIFICING | W (gm.) | AD (mg.) | CHANGE IN W FROM BASAL (gm.) | % CHANGE IN W FROM BASAL | CHANGE IN AD FROM BASAL (mg.) | % CHANGE IN AD FROM BASAL | AD/W |
|---|---|---|---|---|---|---|---|---|---|---|
| X | 1 April | 15 | Starting mash and water *ad libitum* | 144 | 22.0 | — | — | — | — | 0.15 |
| Y | 6 April | 10 | Starting mash and water *ad libitum* | 230 | 33.6 | +84.2 | +58 | +11.6 | +55 | 0.15 |
| Z | 6 April | 15 | Starved for 5 days | 88 | 22.0 | −55.6 | −39 | 0 | 0 | 0.25 |

expense of other tissues. This maintenance of adrenal weight coincident with a loss in body weight is reflected in an AD/W of 0.25, compared with the normal ratio of 0.15. It should be pointed out that there is a fundamental difference in reaction to starvation and to malaria in chicks in that in starvation there is *no actual increase in adrenal weight*, while in malaria there is a marked *increase in adrenal weight*.

## DISCUSSION

Adrenal hypertrophy has been found associated with several pathological conditions: in man, in certain systemic diseases (Sarason, 1943); in pigeons infected with tuberculosis and ascarid worms (Riddle, 1923); and in diseased or injured rats (Donaldson, 1924; Andersen, 1935). There have been several reports of pathological changes in the adrenal cortex of men dying of *P. falciparum* infections (Paisseau and Lemaire, 1916; Natali, 1934; Spitz, 1946; Golden and Overman, 1948) and monkeys dying of *P. knowlesi* (Natali, 1934; Golden and Overman, 1948). To our knowledge adrenal hypertrophy has not been reported for any malarial infection, nor has the increase in adrenal weight relative to body weight been so closely associated with the course of any infection.

There are many factors which are associated with adrenal hypertrophy in addition to infections. These have been reviewed by Tepperman *et al.* (1943). Adrenal hypertrophy is associated with inanition and anoxia. As seen in figure 1 chicks infected with *P. gallinaceum* gain less weight than uninfected chicks. Inanition controls which lost almost 40 per cent of their body weight over a period of 5 days showed no increase in adrenal gland weight. According to Cameron and Carmichael (1946), Mulinos and Pomerantz (1941) and Oleson and Bloor (1941) adrenal hypertrophy in starved animals occurs only when the animals are moribund. It is interesting to note that in chicks infected with *P. gallinaceum* a large increase in adrenal weight occurs only after 50 per cent of the erythrocytes have been invaded and reaches a maximum at the period of greatest rate of death.

Hypertrophy of the adrenals in animals exposed to low oxygen pressure has been reported by Armstrong and Heim (1938) and Langley and Clarke (1942). A severe

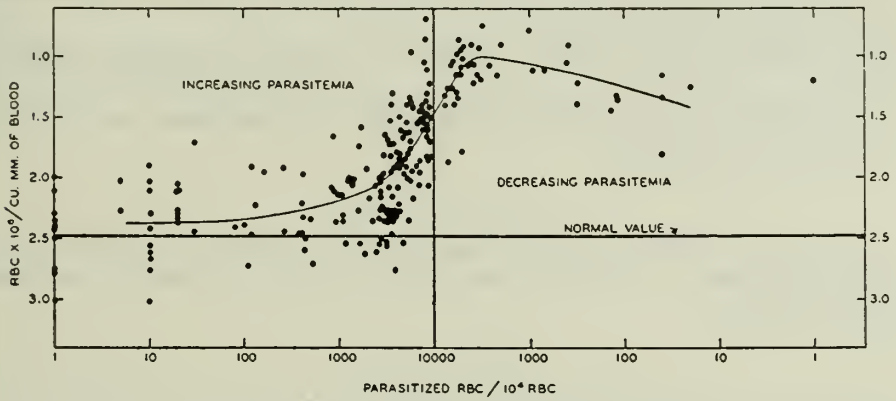


FIG. 5. Changes in the erythrocyte count with varying degrees of parasitemia. The mean erythrocyte count for uninfected chicks is indicated by the horizontal line labelled "normal value."

anemia is produced by *P. gallinaceum* infections in the chick. In *P. lophurae* infections in ducks this anemia is accompanied by severe anoxia (Rigdon and Rorstorfer, 1946; Rigdon and Varnadoe, 1947). In figure 5, the relationship between the course of *P. gallinaceum* infections in the chick and anemia as determined by the concentration of erythrocytes in blood is shown. Anemia increases rapidly after 50 per cent of the erythrocytes have been invaded, and reaches a maximum shortly after peak parasitemia. Additional experiments are necessary before any interpretation can be made on the coincidental maxima of adrenal hypertophy (figure 3) and anemia (figure 5).

There have been a large number of reports of Addison's disease with malaria (generally *P. falciparum*) as etiological agent (Paisseau and Lemaire, 1916; Fraga, 1917; Marañon, 1939; Chessa, 1938; Junior and Brandão, 1937). The data reported in this paper indicate that there are physiological changes occurring during the course of acute, fulminating malaria in the chick which are associated with disturbances in the adrenal gland.

SUMMARY

A study of the adrenal glands of uninfected chicks and chicks infected with $10^3$ erythrocytes parasitized with *Plasmodium gallinaceum* has been made.

1. In uninfected chicks the weight (AD) of fresh adrenals is directly proportional to the body weight (W).

2. In infected chicks, when parasitemia is rising but with less than 50 per cent of the erythrocytes parasitized, there is a slight but significant adrenal hypertrophy.

3. When more than 50 per cent of the erythrocytes are infected, adrenal hypertrophy increases markedly with rising parasitemia. Maximum adrenal hypertrophy is reached about one day after peak parasitemia. The AD/W (mg./gm.) at this time is about 200 per cent higher than in the corresponding uninfected controls.

4. When parasitemia declines further the AD/W of the surviving chicks approaches normal.

5. The adrenal hypertrophy is the result of actual increase in tissue substance and not the result of increase in fluids.

6. Adrenal hypertrophy in malaria cannot be explained on the basis of reduced food intake.

7. The AD/W in chick malaria is at a maximum when the anemia, resulting from the malaria, is at its height.

ACKNOWLEDGMENTS

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# HYPERSENSITIVITY TO SULFADIAZINE OF A CHLORGUANIDE-RESISTANT STRAIN OF *PLASMODIUM GALLINACEUM*

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It has been shown by Greenberg *et al.* (1948) that chlorguanide (Paludrine) and sulfadiazine potentiate each other in their activity against *Plasmodium gallinaceum* in the chick. It is also known that chlorguanide resistance develops quite easily in *P. gallinaceum* exposed to the drug for several serial passages (Williamson, Bertram, and Lourie, 1947; Williamson and Lourie, 1947; Bishop and Birkett, 1947 and 1948). These two facts allow for a closer examination of the mode of interaction of the two drugs. If the mode of action of chlorguanide and sulfadiazine were identical, one might expect that strains of *P. gallinaceum* resistant to chlorguanide would also be resistant to sulfadiazine. This Williamson and Lourie (1947) have shown to be untrue. On the other hand, it is conceivable that the actions of the two drugs might be so related that a chlorguanide-resistant strain might be more sensitive to sulfadiazine. This possibility has been examined and is the subject of the present report.

## METHODS

*Development of chlorguanide resistance.* Week-old New Hampshire Red chicks were inoculated intravenously with $16 \times 10^6$ parasitized erythrocytes from the blood passaged, 8-A strain of *P. gallinaceum* maintained at this laboratory. Immediately after inoculation and again the following morning the chicks were treated orally with chlorguanide hydrochloride, 0.03 mg./gm. body weight. On the eighth day following inoculation of parasites, when between 20 and 40 per cent of the erythrocytes of the chicks were parasitized, the chick with the highest parasite count was selected as donor for the next passage. This passage was treated exactly as the first. In subsequent passages the second dose of the drug was omitted and the donor for the next passage was selected at random. The establishment of some resistance to the drug was indicated by an increase in the 4th day parasite count.

*Assay of drug resistance.* To test for resistance or sensitivity to a drug the technique established for the routine A-1 test (Coatney and Sebrell, 1946) was employed. In this test the chicks are treated twice daily for 4 days beginning 5 to 6 hours before inoculation of $16 \times 10^6$ parasitized erythrocytes (day 0). Parasite counts (parasitized erythrocytes/$10^4$ erythrocytes) are made on the morning of day 4, about 18 hours after the last dose of drug. The minimum effective dose of a drug, by definition, is the smallest dose which causes a 75 per cent reduction in mean parasitemia of 5 treated chicks as compared with 5 to 10 untreated controls. In some of the experiments the response of the parasite to a larger range of dosages was examined than were needed to determine the minimum effective dose of the drug.

## EXPERIMENTAL

The chlorguanide-treated strain was tested for chlorguanide-resistance on the 13th passage (14th week). As seen in table 1 there was definite indication that it required more chlorguanide to suppress the infection in the treated strain than in the parent strain. When retested after the 15th passage (16th week) about eight times as much chlorguanide was required to suppress the parasites of the test strain than of the parent strain. Thereafter the degree of resistance was maintained at about 16- to 32-fold, tests being made only to establish that the resistance to chlorguanide existed without marked change in degree. No attempt was made to increase further the re-

TABLE 1

*The response of the parent and chlorguanide-resistant strains of* Plasmodium gallinaceum *to chlorguanide*

| RESISTANT STRAIN PASSAGE NUMBER | STRAIN TESTED | 4TH DAY PARASITE COUNTS* (PARASITIZED ERYTHROCYTES/$10^4$ ERYTHROCYTES) DOSAGE OF CHLORGUANIDE HCl (MG./GM. BID × 4 DAYS) | | | | | |
|---|---|---|---|---|---|---|---|
| | | .032 | .016 | .008 | .004 | .002 | .001 |
| 13 | resistant | | | | 6800/7003 | 7710/7003 | 7346/7003 |
| | parent | | | | 282/7850 | 2152/7850 | 6210/7850 |
| 15 | resistant | 1256/7120 | 3067/7120 | 8380/7120 | | | |
| | parent | | | | | 4560/5620 | |
| 23 | resistant | 613/7160 | 4610/7160 | | | | |
| | parent | | | | | 15/8190 | |
| 35 | resistant | 123/6800 | 1175/6800 | | | | |
| | parent | | | | | 1628/7280 | 5520/7280 |
| 38 | resistant | 128/5333 | 1500/5333 | | | | |
| | parent | | | | | 1370/6700 | 2040/6700 |
| 41 | resistant | 1/5090 | 1128/5090 | | | | |
| | parent | | | | | 32/2984 | 303/2984‡ |
| 46† | resistant | 3/7300 | 1828/7300 | 4580/7300 | 7000/7300 | 6500/7300 | 7120/7300 |

* Numerator, count of treated chicks; denominator, count of controls.

† Fifth untreated passage.

‡ Count at .0005 mg./gm. b.i.d. 2475/2984.

sistance of the strain to chlorguanide since the minimum effective dose of chlorguanide (0.016 mg./gm.) was nearly equal to the maximum tolerated dose (0.03 mg./gm. b.i.d. × 4 days).

On the 15th passage of the chlorguanide-resistant strain a preliminary trial with sulfadiazine was undertaken (table 2). In this trial, as in all subsequent trials, sulfadiazine was tested against both the chlorguanide-resistant and the parent strain on the same day. Results of this first test indicated that it would require less sulfadiazine to suppress the infection in the resistant than in the parent strain. Retested on the 23rd chlorguanide-resistant strain passage, it required less than one-fourth the amount of sulfadiazine to suppress chlorguanide-resistant parasites as parent strain parasite. On the 35th and 41st passages of the resistant strain it was found to be 8 times more sensitive to sulfadiazine than the parent strain. After the 41st passage, chicks carrying the chlorguanide-resistant strain were no longer treated with

TABLE 2

*The effect of sulfadiazine on a chlorguanide-resistant strain of* Plasmodium gallinaceum

| RESISTANT STRAIN PASSAGE NUMBER | STRAIN TESTED | 4TH DAY PARASITE COUNTS* (PARASITIZED ERYTHROCYTES/$10^4$ ERYTHROCYTES) DOSAGE OF SULFADIAZINE (MG./GM. BID × 4 DAYS) | | | | | |
|---|---|---|---|---|---|---|---|
| | | .0019 | .0037 | .0075 | .015 | .03 | .06 |
| 15 | resistant | | | | | 1/7120 | 8/7120 |
| | parent | | | | | 678/5620 | |
| 23 | resistant | | | 178/7160 | 40/7160 | 10/7160 | |
| | parent | | | | | 818/8190 | |
| 35 | resistant | 5575/6800 | 1810/6800 | | | | |
| | parent | | | | 5010/7280 | 4860/7280 | 242/7280 |
| 41 | resistant | | 5900/5090 | 388/5090 | 702/5090 | 62/5090 | 5/5090 |
| | parent | | | | | 2650/6144 | 316/6144 |
| 46 | resistant | 5460/6880 | 2320/6880 | 2552/6880 | 167/6880 | 16/6880 | 16/6880 |
| | parent | | | 4480/7300 | 3900/7300 | 1850/7300 | 384/7300 |

* Numerator, mean count of 5 treated chicks; denominator, mean count of 5 untreated controls

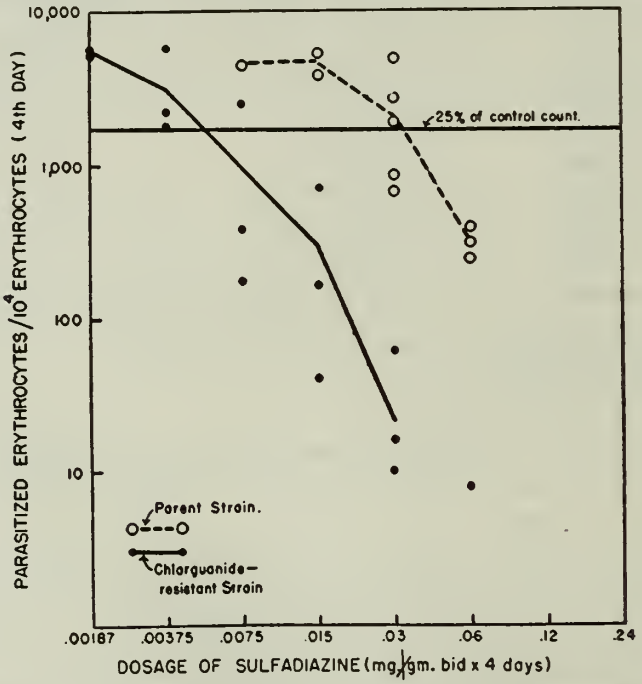


FIG. 1. Response of parent and chlorguanide-resistant strains of *Plasmodium gallinaceum* to sulfadiazine.

the drug. On the 46th passage (5th passage without drug) the strain was still chlorguanide-resistant (table 1), and more than 4 times as sensitive to sulfadiazine as the parent strain. The responses of both strains to various dosages of sulfadiazine are are shown in figure 1.

In view of these findings, it was felt that a re-examination of the activity of other antimalarial drugs against the chlorguanide-resistant parasite was necessary. This was done on the 37th and 38th passage of the chlorguanide-resistant strain. The compounds tested were quinine, chloroquine and pamaquine. There was no difference in activity of these compounds against the resistant or the parent strain of parasite (table 3).

TABLE 3

*The effect of quinine, pamaquine and chloroquine on a chlorguanide-resistant strain of* Plasmodium gallinaceum

| DRUG | DOSAGE (MG./GM. B.I.D.) | 4TH DAY PARASITE COUNT (PARASITIZED ERYTHROCYTES/$10^4$ ERYTHROCYTES) | |
|---|---|---|---|
| | | Parent strain (treated/untreated) | Resistant strain (treated/untreated) |
| Quinine 2 HCl | .005 | 6033/6700 | 4302/5333 |
| | .01 | 5080/6700 | 4267/5333 |
| | .02 | 2994/6700 | 2648/5333 |
| Pamaquine naphthoate | .0005 | 6980/6700 | 5125/5333 |
| | .001 | 1133/6700 | 852/5333 |
| | .002 | 56/6700 | 0/5333 |
| Chloroquine 2 HCl | .001 | 2250/5560 | 2900/5433 |
| | .002 | 43/5560 | 17/5433 |

## DISCUSSION

It has been shown in this report that in *P. gallinaceum* hypersensitivity to sulfadiazine develops coincidentally with chlorguanide resistance. Data presented by Williamson and Lourie (1947) indicate that there may have been hypersensitivity to sulfadiazine in their chlorguanide-resistant strain of *P. gallinaceum*. Knoppers (1947 and personal communication) has obtained a quinine-resistant strain of *P. gallinaceum* which is hypersensitive to pamaquine. The synergism between these compounds was first described by Sinton and Bird (1928), and since amply corroborated by other investigators.

The interaction of sulfadiazine and chlorguanide presents a possible clue as to the mode of action of the latter compound. It may be conjectured that the parasites possess two enzyme systems which accomplish the same end or whose end products are the same. The activity of one of these is hampered by sulfadiazine, the other by chlorguanide. The activity of either system can be reduced by administration of a specifically inhibitory drug or by selecting or "training" parasites to function normally without the enzyme system. The parasite, now relying almost entirely on its alternate enzyme system, would appear to be hypersensitive to the inhibitor of this system.

This hypothesis, which serves to explain all of our findings, fails to explain the results of Bishop and McConnachie (1948). They found that chlorguanide-resistant

strains of *gallinaceum* were resistant to sulfadiazine and vice versa. Their chlorguanide-resistant strain had been exposed almost constantly to the drug for 18 months before it was tested and found to be sulfadiazine-resistant. When they attempted to repeat this result on a strain only recently made chlorguanide-resistant they found that it was not sulfadiazine-resistant. They concluded that chlorguanide and sulfadiazine resistance develop independently. Any integration of the results reported in this paper with those of Bishop and McConnachie will require more investigation.


## SUMMARY

A strain of *P. gallinaceum* in the chick was treated once or twice each passage with chlorguanide hydrochloride, 0.03 mg./gm. body weight.

1. By the 13th passage the strain was chlorguanide-resistant.
2. From the 18th to the 41st passage the treated strain was 16 to 32 times as resistant to chlorguanide as the parent strain.
3. The strain was still chlorguanide-resistant after five untreated passages (41 to 46).
4. On the 15th passage of the chlorguanide-resistant strain it was tested for its reaction to sulfadiazine and found to be hypersensitive to this drug.
5. The chlorguanide-resistant strain was 4 to 8 times as sensitive to sulfadiazine as the parent strain.
6. The chlorguanide-resistant strain was still hypersensitive to sulfadiazine at the 5th untreated (46th) passage.
7. Pamaquine, quinine and chloroquine were equally effective against both the chlorguanide-resistant and the parent strain.


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# MODIFICATION OF *PLASMODIUM GALLINACEUM* INFECTIONS BY CERTAIN TISSUE EXTRACTS

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During unsuccessful attempts to infect various laboratory mammals with the avian malaria parasite, *Plasmodium gallinaceum*, the idea was conceived that the refractoriness of these animals might be associated with the presence in their tissues of some factor capable of inactivating the parasite (Laveran, 1904; Beckman, 1948), and that such a factor might be extracted and perhaps used to protect susceptible animals. The studies reported here show that suitably prepared mammalian tissue extracts did inactivate *P. gallinaceum* to a considerable extent, both in vivo and in vitro, but the fact that extracts with similar activity were prepared from the susceptible chicken as well as from various refractory mammals indicates that the presence of the inactivating substance is not necessarily associated with refractoriness to the parasite.

## INACTIVATING EFFECT ON PLASMODIA IN VITRO

*Extracts of certain pooled mouse tissues.*—The initial experiment was carried out as follows:

The subcutaneous axillary and inguinal lymph nodes, spleens,[1] and bits of liver of two white mice[2] were triturated together and suspended in 15 cc of 0.9 per cent sodium chloride solution. After an hour's settling, the supernatant fluid was used for preparing a suspension of triturated chick embryo brain infected with the exo-erythrocytic forms of *P. gallinaceum* (Haas, Wilcox, Davis and Ewing, 1946), the final proportions being about one part infected brain to 99 parts mouse tissue extract. A similar suspension of infected brain in 0.9 per cent sodium chloride solution was prepared, and the two suspensions were allowed to stand at 4° C. After two hours, and again after 24 hours, the mixtures were used for inoculating chick embryos by the yolk sac method (Haas, Wilcox, Davis and Ewing, 1946); dose: 0.3 cc. The results, summarized in table 1, indicate appreciable inactivation of the malaria parasites by the tissue extract in both instances.

A second pilot experiment, allowing two hours of contact at room temperature,

[1] There have been a number of investigations on the activity of spleen and leucocyte extracts against various infectious agents and in various diseases, including malaria. The studies reported in this presentation were not based on these earlier investigations, nor did they develop along similar lines. For reference purposes, however, some of these reports of other workers have been included in the bibliography (Hiss and Zinsser, 1909; Nutini and Kreke, 1942; Nutini et al., 1945; Thomas and Nutini, 1947; White, 1943, 1944).

[2] The mice (*Mus musculus*) used were descendants of the stock NIH strain, produced in a colony maintained at the Memphis Laboratory since December, 1944.

and using the chorio-allantoic membrane route of inoculation (Zuckerman, 1946), dose: 0.5 cc, gave confirmatory results, also shown in table 1.

Similar inactivation of exo-erythrocytic forms was demonstrated in a third experiment, using chick brains infected with these parasites, suspended in tissue extract in the manner described for the chick embryo experiments, and inoculated into

TABLE 1

*Inactivating effect on various stages of* Plasmodium gallinaceum *following contact in vitro with extract of pooled spleen, lymph nodes, and liver of the white mouse*

Infection determined in chick embryos as described by Haas, Wilcox, and Ewing (1945), in brain-inoculated chicks as described by Haas, Wilcox, Davis, and Ewing (1946), and in other chicks by daily* blood examinations for 3 weeks.

| EXPERIMENT NUMBER | TEST ANIMAL | INFECTIOUS INOCULUM | CONTACT IN VITRO OF INFECTIOUS INOCULUM AND DILUENT | INFECTIOUS INOCULUM SUSPENDED IN | | | |
|---|---|---|---|---|---|---|---|
| | | | | Pooled mouse tissue extract | | 0.9 per cent sodium chloride solution | |
| | | | | Animals | | Animals | |
| | | | | Inoculated | Infected | Inoculated | Infected |
| 1 | Chick embryo | Chick embryo e.e. brain | 2 hrs.—4° C | 12 | 4 | 14 | 9 |
| | Chick embryo | Chick embryo e.e. brain | 24 hrs.—4° C | 17 | 0 | 16 | 7 |
| 2 | Chick embryo | Chich embryo e.e. brain | 2 hrs.—room temp. | 18 | 2 | 9 | 9 |
| 3 | Chick | Chick e.e. brain | 2 hrs.—room temp. | 18 | 1 | 14 | 13 |
| 4(a) | Chick | Triturated mosquitoes | 1 hr.—room temp. 5 hrs. ice bath | 8 | 0 | 12 | 6 |
| 4(b) | Chick | Hemolyzed, parasitized chick erythrocytes | 3 hrs.—room temp. | 18 | 0 | 20 | 19 |

* In this report, daily does not include Saturday and Sunday.

chicks[3] (Haas, Wilcox, Davis and Ewing, 1946) after standing two hours at room temperature; dose: 0.5 cc intramuscularly (table 1).

A subsequent experiment indicated inactivation of sporozoites and erythrocytic forms of *P. gallinaceum:*

(a) Mosquitoes (*Aedes egypti*) heavily infected with sporozoites of the regular passage strain (Haas, Wilcox, Laird, Ewing and Coleman, 1948) were triturated in the manner described by Coatney, Cooper and Trembley, (1945) except that suspensions were prepared to contain an estimated equivalent of three infected mos-

[3] All chicks used in these experiments were 9–12 day old (unless otherwise stated) white Wyandottes purchased from a local dealer.

quitoes per 0.5 cc, and the suspending fluid was mouse tissue extract plus 5 per cent normal chick blood (Coggeshall, Porter and Laird, 1944) for one group of mosquitoes, and 0.9 per cent saline plus 5 per cent normal chick blood for the other. The suspensions stood one hour at room temperature, and four hours in an ice bath, and were then used to inoculate two groups of chicks, the dose being 0.5 cc intramuscularly. The results, summarized in table 1 (4a) indicate inactivation of the sporozoites by the tissue extract. (This single, unrepeated experiment was a pilot study only, as were the other in vitro tests described; no conclusions were drawn, except that further study was indicated. This was carried out as described later.)

(b) Pooled heparinized blood from chicks infected with blood passage strain *P. gallinaceum* (Haas, Wilcox, Laird, Ewing and Coleman, 1948) with a mean parasite density of 60 per cent parasitized erythrocytes, was hemolyzed with serum from rabbits previously inoculated with repeated doses of normal chick erythrocytes. The hemolyzed, parasitized erythrocytes were packed by centrifugation, and then resuspended in either mouse tissue extract or in 0.9 per cent saline, the quantity of suspending fluid in each case being about four times the original plasma volume.[4] After three hours at room temperature, with frequent agitation, the suspensions were used to inoculate two groups of chicks intramuscularly, the dose being 0.3 cc. Results, summarized in table 1 (4b) indicate apparently complete inactivation of the parasites by the tissue extract.

*Extracts of individual tissues of various animals.*—When extracts of lymph nodes, liver, and spleen of the white mouse were tested separately against the exo-erythrocytic forms of *P. gallinaceum*, in a manner identical with that used for the extract of the same tissues pooled (pieces of liver, and one or more lymph nodes were cut to approximately the size of the spleen for these tests), as indicated in table 2, the extract of spleen gave complete inactivation. The failure of separate lymph node and liver extracts to produce inactivation in this experiment was not further investigated, since the objective was to narrow down subsequent investigations to a preparation reasonably certain to be effective, rather than to compare relative activity of various tissues.

The evidence of some inactivating effect by extract of chicken spleen is shown in table 2 (a piece of spleen from a 4 week old chicken, approximately equal in size to the mouse spleen, was used); its significance in altering the original concept from which these studies arose has already been alluded to.

Extracts of spleens (made from pieces approximately equal in size to mouse spleen) of the white rat, cotton rat, and rice rat[5] were tested, in the manner previously de-

[4] This process, in detail, was as follows: 6 cc pooled, heparinized chick blood was centrifuged; plasma was discarded, and replaced with 0.9 per cent saline. Into a fresh centrifuge tube was placed 3 cc serum from a rabbit immunized against chick erythrocytes, 3 cc normal rat serum (to lyse chick erythrocyte nuclei), and 3 cc Lyovac 1/10. The erythrocyte-saline suspension was placed in this tube and thoroughly shaken. After 15 minutes at room temperature, the contents of the tube were divided into two equal portions, put into each of two centrifuge tubes, and centrifuged 15 minutes. Supernatant fluid was discarded, and tissue extract or saline added to the sediment in sufficient quantity to bring volume in each tube up to 3 cc. After thorough shaking 9 cc more of tissue extract or saline was added to the appropriate tube. This was the final mixture used for inoculation.

[5] All these "rats" were from NIH stock. The cotton rat is *Sigmodon hispidus*; the rice rat is *Oryzomys palustris*.

scribed, against erythrocytic forms of *P. gallinaceum* in an experiment summarized in table 3; all produced an appreciable inactivating effect.

In another experiment, also summarized in table 3, extract of the pulp of cow spleen (from a piece about the size of a mouse spleen) inactivated the erythrocytic forms of *P. gallinaceum*, while extract of pig spleen pulp gave partial inactivation, comparable to that given by chick spleen (table 2).

TABLE 2

*Effect upon exoerythrocytic forms of* P. gallinaceum *of contact with separate extracts of spleen, lymph nodes, and liver of the white mouse, and extract of spleen of the chicken*

| EMULSION OF CHICK BRAINS INFECTED WITH EXOERYTHROCYTIC FORMS OF *P. gallinaceum* SUSPENDED IN FLUID INDICATED (DILUTION 1:100) | EMULSIONS OF INFECTED BRAINS INOCULATED INTO CHICKS AFTER STANDING 2 HOURS AT ROOM TEMPERATURE | |
|---|---|---|
| | Chicks | |
| | Inoculated | Infected |
| Extract of mouse spleen | 16 | 0 |
| Extract of mouse lymph nodes | 5 | 4 |
| Extract of mouse liver | 15 | 15 |
| Extract of chicken spleen | 8 | 4 |
| 0.9 per cent sodium chloride solution | 9 | 9 |

TABLE 3

*Inactivating effect on erythrocytic forms of* P. gallinaceum *shown by saline extracts of spleens of various animals, during contact in vitro*

All chicks examined by daily blood smears for 3 weeks, or until parasitemia was proved

| EXPERIMENT NUMBER | HEMOLYZED, PARASITIZED CHICK ERYTHROCYTES SUSPENDED IN EXTRACT OF SPLEEN OF ANIMAL INDICATED | SUSPENSIONS OF PARASITIZED CELLS INOCULATED INTO CHICKS AFTER STANDING 3 HRS. AT ROOM TEMPERATURE | |
|---|---|---|---|
| | | Chicks | |
| | | Inoculated | Infected |
| 1 | White rat | 8 | 1 |
| | Cotton rat | 15 | 2 |
| | Rice rat | 6 | 0 |
| | Saline control | 8 | 5 |
| 2 | Cow | 13 | 0 |
| | Pig | 17 | 9 |
| | Saline control | 14 | 14 |

Because extract of bovine spleen appeared to give satisfactory inactivation, and such spleens were obtainable in sufficient quantity to meet all experimental needs, subsequent studies on spleen extracts were confined to bovine spleens.[6] In 12 experiments with such extracts, employing simple preparations of from 7.5 to 20 grams of bovine spleen per 100 cc of 0.9 per cent saline, (solid material was removed by centrifuging 30 minutes at 1200[7] r.p.m.) a considerable degree of inactivation of the erythrocytic forms of *P. gallinaceum* during contact of 3 hrs. at room temperature

[6] We are grateful to the Abraham Bros. Packing Co., and to the Memphis Packing Co., both of Memphis, Tenn., for supplying these spleens free of charge, in whatever quantity we requested.

[7] International Centrifuge No. 2 was used, unless otherwise specified.

TABLE 4

*Inactivating effect on erythrocytic forms of* P. gallinaceum *shown by saline extracts of bovine spleen, during contact in vitro*

Summary of results in 12 experiments. All chicks examined by daily blood smears for 3 weeks or until parasitemia was proved.

| EXPERIMENT NUMBER | SUSPENSIONS OF HEMOLYZED, PARASITIZED CELLS INOCULATED INTO CHICKS AFTER STANDING 3 HRS. AT ROOM TEMPERATURE | | | |
|---|---|---|---|---|
| | In spleen extract | | In 0.9 per cent saline | |
| | Chicks | | Chicks | |
| | Inoculated | Infected | Inoculated | Infected |
| 1 | 12 | 0 | 14 | 14 |
| 2 | 28 | 2 | 30 | 30 |
| 3 | 16 | 3 | 23 | 20 |
| 4 | 16 | 10 | 18 | 18 |
| 5 | 16 | 2 | 16 | 15 |
| 6 | 16 | 5 | 19 | 16 |
| 7 | 15 | 9 | 19 | 14 |
| 8 | 45 | 11 | 17 | 14 |
| 9 | 50 | 12 | 15 | 10 |
| 10 | 26 | 10 | 6 | 5 |
| 11 | 76 | 16 | 17 | 17 |
| 12 | 51 | 16 | 19 | 14 |
| Total | 367 | 96 | 213 | 187 |
| Per cent | — | 26 | — | 87 |

TABLE 5

*Inactivating effect on exoerythrocytic forms of* P. gallinaceum *shown by saline extracts of bovine spleen during contact in vitro*

Summary of results in 5 experiments.

| EXPERIMENT NUMBER | ANIMAL USED FOR TEST | EMULSIONS OF INFECTED BRAINS INOCULATED AFTER STANDING 3 HRS. AT ROOM TEMPERATURE | | | |
|---|---|---|---|---|---|
| | | In Spleen Extract | | In Saline | |
| | | Inoculated | Infected | Inoculated | Infected |
| 1 | Chick | 16 | 10 | 18 | 18 |
| 2 | Chick Embryo | 29 | 2 | 16 | 16 |
| 3 | Chick Embryo | 12 | 7 | 10 | 9 |
| 4 | Chick | 45 | 21 | 25 | 14 |
| 5 | Chick | 37 | 30 | 15 | 14 |
| Total | — | 139 | 70 | 84 | 71 |
| Per cent | — | — | 50 | — | 83 |

was observed, as indicated in table 4. Against the exo-erythrocytic parasites, however, the inactivating effect, though it occurred, was appreciably less than against the erythrocytic forms, as shown in table 5.

Smears made from exo-erythrocytic and erythrocytic forms after contact with the various tissue extracts in vitro revealed no detectable morphological effect upon the parasites in any of these experiments.

### MODIFICATION OF INFECTION IN VIVO

*Extracts of bovine spleen.*—The experiments on inactivation in vitro were regarded as preliminary to the real aim of these studies, which was to produce an extract capable of protecting chicks against an actual *P. gallinaceum* infection. The crude extracts used to demonstrate inactivation in vitro did not suffice for this purpose, and subsequent efforts were directed toward production of a more concentrated product. Concentration was accomplished as follows:

Fresh bovine spleens were frozen and kept overnight (or longer) at approximately 0° C. When thawed for use, they were decapsulated with a sharp knife. Extraction was done with distilled water or 0.9 per cent saline (either seemed satisfactory) by grinding splenic pulp and the diluent in a Waring blender: 40 grams of pulp to 150 cc diluent. The suspension stood in a refrigerator 1½ hrs. and was then centrifuged at 1200 r.p.m. for 30 minutes; the supernatant fluid was decanted for use and the sediment discarded. The fluid thus obtained was chilled to 0° C and cold ethyl alcohol (all alcohol used in these experiments was U.S.P.) was added, 150 cc alcohol to 450 cc extract; this addition was done drop-by-drop from a separatory funnel, at 0° C. After standing 1½ hrs., the precipitate which formed was separated by centrifuging at 1200 r.p.m. for 30 minutes and was kept frozen until used.

The method of administration was to suspend the precipitate in water equal to 1/10 the volume of that used to extract the spleen (thus concentration was approximately 10 times). Each chick received subcutaneously 1 cc of such a suspension on the day prior to inoculation with parasites, 1 cc three times daily on the day of parasite inoculation, and 1 cc three times daily on each of the next two days. Control chicks received 0.9 per cent sodium chloride solution on the same schedule.

The standard parasite inoculum consisted of triturated sporozoite-infected mosquitoes suspended in serum-saline (Coatney, Cooper and Trembley, 1945), each chick receiving the equivalent of 2 infected mosquitoes, by subcutaneous injection.

The sample protocol shown in table 6 indicates the extent to which protection of chicks against *P. gallinaceum* infection could be attained; in this particular experiment, 6 of the 12 treated chicks failed to develop microscopically detectable parasitemia within the 3 weeks' period of observation, whereas all the controls (treated with saline solution) showed parasites in the blood by the 13th day. It is also evident from table 6 that the parasitemia appeared and developed more rapidly in the control group.

Similar results were obtained in the 11 experiments summarized in table 7: there was delay in development of the infection, and only 64 per cent of the treated chicks showed parasites on blood smears during the period of observation, as compared with 97 per cent of controls.

Unfortunately, the concentrated spleen extract as prepared in these experiments was toxic to the chicks. Treated chicks developed necrosis and sometimes gangrene at the site of inoculation; in some experiments many died or had to be killed because

TABLE 6

*Modification of sporozoite-induced* P. gallinaceum *infection by concentrated extract of bovine spleen*

Chicks 1–12 treated with spleen extracts; chicks 13–24 given saline only. All chicks given equivalent of 2 sporozoite-infected mosquitoes (Coatney, Cooper, and Trembley, 1945).

| CHICK NUMBER | PARASITIZED ERYTHROCYTES PER 10,000 RED BLOOD CELLS ON INDICATED POST-INOCULATION DAY* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 8 | 9 | 12 | 13 | 14 | 15 | 16 | 17 | 20 | 21 |
| 1 | 0 | 0 | 0 | 20 | 50 | 630 | 8500 | | | |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 10 | 70 | 450 | 1800 | | | |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 5 | 10 | 2700 | 3600 | | |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 3300 | 4800 | | |
| 10 | 0 | 0 | 20 | 90 | 330 | 1300 | | | | |
| 11 | 0 | 0 | 5 | 10 | 30 | 360 | 3200 | | | |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 5 | 5 | 40 | | | | | |
| 14 | 0 | 0 | 5 | 30 | 50 | | | | | |
| 15 | 0 | 5 | 50 | 1400 | 2800 | | | | | |
| 16 | 0 | 0 | 20 | 40 | 310 | | | | | |
| 17 | 0 | 0 | 5 | 20 | 30 | | | | | |
| 18 | 0 | 0 | 30 | 250 | 1600 | | | | | |
| 19 | 0 | 5 | 3500 | 4000 | | | | | | |
| 20 | 0 | 0 | 0 | 10 | 20 | | | | | |
| 21 | 0 | 0 | 1000 | 1900 | 3500 | | | | | |
| 22 | 0 | 0 | 0 | 20 | 40 | | | | | |
| 23 | 0 | 0 | 70 | 210 | 1500 | | | | | |
| 24 | 0 | 0 | 40 | 170 | 110 | | | | | |

* Examinations were discontinued when presence of infection was definite. Only negative chicks were examined for the full 21-day period.

TABLE 7

*Modification of sporozoite-induced* P. gallinaceum *infection by concentrated extract of bovine spleen*

Summary of 11 experiments: 170 treated chicks and 146 controls. Each chick received equivalent of 2 infected mosquitoes (Coatney, Cooper, and Trembley, 1945).

| DAY AFTER MOSQUITO INOCULATION | CUMULATIVE PERCENTAGE OF CHICKS WITH MICROSCOPICALLY PATENT PARASITEMIA BY DAY INDICATED | |
|---|---|---|
| | 170 treated chicks | 146 control chicks |
| 8 | 3 | 20 |
| 9 | 12 | 37 |
| 12 | 46 | 87 |
| 13 | 55 | 94 |
| 14 | 59 | 97 |
| 15 | 61 | 97 |
| 16 | 62 | 97 |
| 17 | 63 | 97 |
| 20 | 64 | 97 |
| 21 | 64 | 97 |

of these reactions. Attempts to produce a potent extract without this toxicity were unsuccessful.

*Extracts of bovine blood.*—The spleen extracts contained large quantities of finely suspended solid matter which could not be removed either by filtration, high-speed centrifugation, or treatment with weak acids or alkalis, or with organic solvents. Since it was believed that the toxic effects—particularly the extensive local irritation—might be due in considerable part to this solid material, a tissue other than spleen was tried as an alternative; bovine blood was the tissue used.[8]

Initially, bovine blood was extracted as follows:

Several liters of fresh blood was allowed to clot and about 2 liters of the serum-cell mixture capable of being freed from the clot was removed by suction and frozen. On the following morning it was thawed and treated with ethyl alcohol at 0° C, drop-by-drop, 150 cc alcohol to 450 cc serum-cell mixture. Resulting precipitate separated by centrifuging at 1200 r.p.m. for 30 minutes, taken up in about 150 cc distilled water, and then lyophilized[9] to dryness.

For inoculation in the initial experiment, the lyophilized residue obtained from 2,000 cc of serum-cell mixture was suspended in 150 cc distilled water and chicks were given the same dosage and schedule as for spleen extract. In this experiment, shown in table 8, parasites were found in the blood of 8 out of 14 treated chicks during the 3 weeks observation period, as compared with all of 10 controls. It is also clear from this table that parasitemia tended to appear later in the treated group.

An alcohol precipitate of serum, freed from cells by centrifuging, employed in the same dosage as the extract from the serum-cell mixture, produced no effect on the infection in chicks. When citrated blood (7 grams crystalline sodium citrate per 4 liters of blood) was used, a concentrated alcohol precipitate from the plasma likewise gave no effect. When it thus became evident that activity of the type shown in table 8 originated in the cells rather than the fluid portions of the blood, subsequent experiments were carried out with cells separated by centrifuging, either from serum—where clotting was permitted—or from plasma—where the blood had been citrated.

The method of extracting blood cells finally adopted was as follows:

Either whole citrated blood, or the serum-cell mixture freed from clotted blood by retraction of the clot, was put through a Sharples Super-Centrifuge (laboratory model, electric, open type, blood separator bowl). This was done within 1–2 hours after blood had been taken from the donor cattle. The cells thus separated were frozen and kept at 0° C until treated further, either the next day or 2–3 days later. They were then thawed, suspended in an equal volume of distilled water, and kept in a refrigerator for $1\frac{1}{2}$ hours. The mixture was chilled to 0° C, and treated with alcohol in the usual manner in the cold: 150 cc alcohol to 450 cc cell-water mixture. The resulting precipitate, after $1\frac{1}{2}$ hrs. standing at 0° C, was separated by the Sharples

[8] We are grateful to the Abraham Bros. Packing Co., of Memphis, Tenn., for supplying without cost to us all bovine blood used in these experiments.

[9] We are indebted to Dr. Jack R. Leonards, of the Department of Clinical Biochemistry, School of Medicine, Western Reserve University, for instructing us in the method of lyophilizing appreciable quantities of the type of materials used in these experiments.

(standard clarifier bowl). It was then suspended in just enough distilled water to permit its being readily poured, and was lyophilized to dryness. With this method, the yield was generally about 1 gram of final residue from 100 cc of blood cells. (There was some variation in this yield, depending upon method of handling. With the regular laboratory centrifuge, 1200 r.p.m. for 30 minutes sometimes gave twice the yield that was obtained from the Sharples; passing the material twice through

TABLE 8

*Modification of sporozoite-induced* P. gallinaceum *infection by concentrated extract of bovine blood*

Chicks 1–14 treated with blood extract; chicks 15–24 given saline only. All chicks given equivalent of 2 sporozoite-infected mosquitoes (Coatney, Cooper and Trembley, 1945).

| CHICK NUMBER | PARASITIZED ERYTHROCYTES PER 10,000 RED BLOOD CELLS ON INDICATED POST-INOCULATION DAY* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 8 | 9 | 12 | 13 | 14 | 15 | 16 | 17 | 20 | 21 |
| 1 | 0 | 0 | 360 | 500 | 180 | | | | | |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 1900 | 5500 | | | | | | |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 5 | 250 | 2700 | 4400 | | | | | |
| 9 | 0 | 0 | 30 | 60 | 260 | | | | | |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 290 | | |
| 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 5 | 5 | 0 | 10 | 1200 | 4600 | 5500 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 1600 | 2100 | 2600 |
| 14 | 0 | 0 | 5 | 10 | 30 | | | | | |
| 15 | 0 | 0 | 3500 | 4600 | | | | | | |
| 16 | 0 | 0 | 280 | 330 | | | | | | |
| 17 | 0 | 5 | 2200 | 5800 | | | | | | |
| 18 | 0 | 0 | 20 | 50 | | | | | | |
| 19 | 0 | 0 | 360 | 2900 | | | | | | |
| 20 | 0 | 100 | 5000 | | | | | | | |
| 21 | 5 | 10 | 3400 | 2900 | | | | | | |
| 22 | 0 | 0 | 20 | 40 | | | | | | |
| 23 | 0 | 0 | 40 | 90 | | | | | | |
| 24 | 10 | 30 | 5000 | 3800 | | | | | | |

* Examinations were discontinued when presence of infection was definite. Negative chicks were examined for the full 21-day period.

the Sharples increased the yield. Even with lesser yield per unit volume, separation by the Sharples was more satisfactory because so much greater quantities could be handled in a given period.)

The dosage was 0.5 grams of lyophilized residue per chick, suspended in 3 cc distilled water, and given in three injections of 1 cc each. This dosage was given on the day of mosquito inoculation, and the first, second, fifth, sixth and seventh days after mosquito inoculation. Larger doses were too toxic, and smaller ones had less influence on the infection.

In 9 experiments, summarized in table 9, treated chicks developed parasitemia later, and less of them (79 per cent) showed parasites in the blood during the 3 weeks observation than did the controls (93 per cent).

Thus, the type of anti-plasmodial activity noted in concentrated spleen extract was also shown by the extract similarly prepared from blood cells. Whether the activity in the spleen preparation was derived from the copious supply of blood cells contained in that organ cannot be determined from the data available from these experiments. Nor can it be concluded that the activity in the blood cell extract is derived from either red cells or white cells alone. The extracts used have contained extractives from all the elements included in the cellular fraction separated by the centrifuge.

TABLE 9

*Modification of sporozoite-induced* P. gallinaceum *infection by concentrated extract of bovine blood cells*

Summary of 9 experiments: 162 treated chicks and 98 controls. Each chick received equivalent of 2 infected mosquitoes (Coatney, Cooper, and Trembley, 1945).

| DAY AFTER MOSQUITO INOCULATION | CUMULATIVE PERCENTAGE OF CHICKS WITH MICROSCOPICALLY PATENT PARASITEMIA BY DAY INDICATED | |
|---|---|---|
| | 162 treated chicks | 98 control chicks |
| 8 | 1 | 9 |
| 9 | 5 | 22 |
| 12 | 46 | 90 |
| 13 | 52 | 93 |
| 14 | 58 | 93 |
| 15 | 60 | 93 |
| 16 | 68 | 93 |
| 17 | 71 | 93 |
| 20 | 79 | 93 |
| 21 | 79 | 93 |

The toxicity which made spleen extract objectionable was also evident in the experiments with blood extract, but to a lesser degree. By using chicks 14–16 days old, it was usually possible to avoid serious loss from this cause.

*Extract of chicken blood.*—As indicated previously, some inactivation of *P. gallinaceum* in vitro was shown by an extract of chicken spleen. In a subsequent experiment, summarized in table 10, an extract prepared from chicken blood cells (the chickens were 4–5 weeks old) in the same manner as that from bovine cells gave appreciable protection against the parasite, again indicating that the presence of the inactivating substance is not dependent upon refractoriness.

## CHARACTERIZATION OF ACTIVE PRINCIPLE

In order to obtain the active principle of bovine blood extract in purer form, the following procedure was carried out:

Lyophilized alcohol precipitate prepared from bovine blood cells by the method previously described, and weighing 44 grams, was suspended in 250 cc cold distilled water, and allowed to stand 1½ hrs. in the refrigerator, with repeated shaking. The suspension was then centrifuged for 30 minutes at 1200 r.p.m., and the supernatant

fluid set aside. The sediment was re-suspended in 150 cc distilled water, and allowed to stand as before. Again the mixture was centrifuged, and the supernatant fluid pooled with that from the first treatment. Suspension of the sediment was then carried out a third time, using 150 cc of distilled water, and treating as before. This third supernatant was added to the others. Sediment was then discarded; the supernatant fluid was lyophilized, yielding 23 grams of residue. This residue was treated with 200 cc cold ether (anesthetic, U.S.P.), for two hours in the refrigerator;

TABLE 10

*Modification of sporozoite-induced* P. gallinaceum *infection by concentrated extract of chicken blood cells*

Chicks 1–9 treated with extract; chicks 10–19 given saline only. All chicks given equivalent of sporozoite-infected mosquitoes (Coatney, Cooper, and Trembley, 1945).

| CHICK NUMBER | PARASITIZED ERYTHROCYTES PER 10,000 RED BLOOD CELLS ON INDICATED POST-INOCULATION DAY* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 8 | 9 | 12 | 13 | 14 | 15 | 16 | 17 | 20 | 21 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 10 | 10 | 110 | 500 | 2700 | 2800 | 2700 | 1600 |
| 3 | 0 | 0 | 20 | 120 | 190 | 220 | 3000 | 1600 | 2000 | 280 |
| 4 | 0 | 0 | 20 | 40 | 1800 | 3700 | 5100 | 4400 | 2400 | 2900 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 10 | 130 | 5700 | 6900 | 2100 |
| 10 | 90 | 2700 | 2000 | 1800 | 2500 | 3400 | | | | |
| 11 | 5 | 1000 | 3700 | 6800 | 3800 | 4500 | | | | |
| 12 | 0 | 110 | 1300 | 1600 | 6000 | 3900 | | | | |
| 13 | 5 | 2600 | 5400 | 7300 | 4600 | 4200 | | | | |
| 14 | 40 | 500 | 140 | 10 | 0 | 5 | | | | |
| 15 | 30 | 6500 | 4400 | 3900 | 3700 | 4500 | | | | |
| 16 | 10 | 280 | 2500 | 2000 | — | — | | | | |
| 17 | 30 | 3400 | 3500 | — | — | 5100 | | | | |
| 18 | 10 | 1000 | — | 150 | 60 | 10 | | | | |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* Examinations were discontinued in some cases after infection was definite. Negative chicks were examined for the full 21-day period.

the resulting mixture was centrifuged at 1200 r.p.m. for 10 minutes and the supernatant fluid (containing the ether-soluble fractions and showing a pale yellow color) discarded. The sediment was suspended in 200 cc cold distilled water, allowed to stand 3 hrs. in the refrigerator, with shaking, and then centrifuged at 1200 r.p.m. for 20 minutes. The solid material rose to the surface of the centrifuge tubes during this process; this was discarded and the underlying fluid was lyophilized, yielding 13.3 grams of residue.

This process reduced the solid residue remaining after final lyophilization from an original 44 grams to 13.3 grams, and presumably eliminated the ether-soluble components. The residue was tested against *P. gallinaceum* in chicks, using the same

schedule previously described for blood cell extract, except that the dose was reduced from 0.5 grams to 0.18 grams per day ($13.3/44 \times 0.5 = 0.15$; the 0.18 used was thus slightly more generous than strict calculation on the basis of reduction in weight of the residue required). Appreciable modification of the infection occurred, as shown in table 11, although it was less marked than that experienced with the cruder extracts (tables 8 and 9).

TABLE 11

*Modification of sporozoite-induced* P. gallinaceum *infection by material derived from bovine blood cells, repeatedly extracted with water and subsequently treated with ether*

Chicks 1–11 treated with extract; chicks 12–22 given saline only. All chicks given equivalent of 2 sporozoite-induced mosquitoes (Coatney et al., 1945).

| CHICK NUMBER* | PARASITIZED ERYTHROCYTES PER 10,000 RED BLOOD CELLS ON INDICATED POST-INOCULATION DAY | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 8 | 9 | 12 | 13 | 14 | 15 | 16 | 17 | 20 | 21 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 50 | 160 | 1100 | | | | |
| 5 | 0 | 0 | 10 | 5 | 10 | 160 | | | | |
| 6 | 0 | 0 | 10 | 180 | 1800 | 1400 | | | | |
| 7 | 0 | 0 | 10 | 130 | 1400 | 1000 | | | | |
| 8 | 0 | 0 | 20 | 120 | 1800 | 5100 | | | | |
| 9 | 0 | 0 | 40 | 170 | | | | | | |
| 10 | 0 | 0 | 90 | 1100 | 4800 | 7400 | | | | |
| 11 | 0 | 0 | 1400 | 4800 | 6700 | 7600 | | | | |
| 12 | 0 | 0 | 40 | 420 | 1900 | 4200 | | | | |
| 13 | 0 | 0 | 50 | 390 | 1200 | 2400 | | | | |
| 14 | 0 | 0 | 630 | 1900 | 2200 | 4600 | | | | |
| 15 | 0 | 0 | 1100 | 1900 | 4600 | 6900 | | | | |
| 16 | 0 | 0 | 2300 | 3400 | 4100 | 5400 | | | | |
| 17 | 0 | 0 | 2300 | 3500 | 3200 | 180 | | | | |
| 18 | 0 | 0 | 2300 | 4200 | 4000 | 5500 | | | | |
| 19 | 0 | 0 | 2400 | 2400 | 2500 | 170 | | | | |
| 20 | 0 | 10 | 1500 | 3500 | 4700 | 6800 | | | | |
| 21 | 0 | 10 | 2200 | 5000 | 4400 | 1400 | | | | |
| 22 | 0 | 30 | 2100 | 30 | 0 | 20 | | | | |

* Order of chicks in table arranged to facilitate comparison of treated and control groups.

In another experiment, lyophilized alcohol precipitate was subjected to the repeated water extraction described above, but not to ether treatment. The residue was suspended in just enough distilled water to allow pouring, placed in a Visking dialyzing membrane, and immersed in distilled water for 42 hours in the refrigerator. The material inside the bag, and the fluid outside, were lyophilized separately and each used to inoculate chicks according to the standard schedule, but with dosages reduced in the manner indicated in the preceding paragraph. The infection in the chicks treated with the heavier material inside the bag was modified in the usual way; that in the chicks given the dialyzed portion showed no such modification.

Further identification of the active principle in these extracts must await additional

investigations, which are now in progress. The data presented above indicate that: (1) it is present in water extracts suitably prepared; (2) it is precipitated from such extracts by ethyl alcohol when used in the proper proportions; (3) it remains with the insoluble residue after ether treatment; and (4) it fails to pass through a Visking dialyzing membrane during 42 hours at 4° C. These observations suggest that the active factor either is a protein, or is closely associated with the protein components of the extract.

The derivation of an anti-plasmodial factor from blood cells, as described above, suggests consideration of the hypothesis advanced by H. R. Jacobs (1947). This is that an animal infected with malaria tends to destroy its own (unparasitized) erythrocytes, and that this phenomenon represents a defense activity. Presumably, substances liberated by this erythrocytic destruction may have some deleterious effect—direct or indirect—on the parasite. The background for this reasoning is thoroughly explored in his presentation. Jacobs was unable to support this hypothesis by experimental evidence, but his animals (ducks infected with *P. lophurae*) were treated with blood or blood derivatives which were far more dilute than the concentrated extracts used in the studies reported here.

The observations detailed in this report indicate that a substance obtained during extraction of erythrocytes (and also during similar extraction of the spleen) was capable of producing the sort of effect that Jacobs postulated, but whether that substance was present in an active form in the natural state of these tissues, or whether it originated during the process of extraction, cannot be clearly determined from the data thus far available. Tissue extracts capable of exerting harmful activity against micro-organisms (Nutini et al., 1945; Bloom and Blake, 1948), and extracts capable of furthering the survival of micro-organisms (Nutini et al., 1945; Whitman, 1948) have been described. Since the methods of preparation were different from those used in the studies reported here, comparison between the present product and others of the type referred to is not practicable.

## SUMMARY

1. In preliminary experiments, saline extracts of pooled lymph nodes, spleen, and liver of the white mouse produced an inactivating effect upon the sporozoites, exo-erythrocytic stages, and erythrocytic forms of *P. gallinaceum* after suitable contact in vitro.

2. Similar extracts of each tissue, tested separately against exo-erythrocytic forms, showed inactivation only by the extract of spleen.

3. Extracts of spleens of the white rat, rice rat, cotton rat, and cow, tested against erythrocytic stages, all showed inactivating effect. Extracts of spleens of the chicken and of the pig acted similarly; they appeared somewhat less potent than extracts of the rat and cow spleens, but the tests were not quantitatively accurate in this respect.

4. There were 367 chicks inoculated with erythrocytic forms of *P. gallinaceum* which had been subjected to contact with extract of bovine spleen: 26 per cent of them developed microscopically detectable parasitemia during 3 weeks' observation. There were 213 controls: 87 per cent developed parasitemia.

5. Activity of the bovine spleen extract against exo-erythrocytic stages was less pronounced: 50 per cent of chicks or chick embryos inoculated with parasites which had been in contact with the extract developed detectable infection, as compared with 83 per cent of controls.

6. Bovine spleen extract, concentrated by ethyl alcohol precipitation, modified sporozoite-induced *gallinaceum* infection in vivo. In 11 experiments, there were 170 treated chicks: 64 per cent developed patent infections during 3 weeks' observation; there were 146 controls, of which 97 per cent developed infections. In treated chicks, parasitemia tended to develop more slowly than in controls.

7. Bovine spleen extract was toxic to chicks and its use was therefore discontinued. Extracts of bovine blood cells, similarly prepared and concentrated, were found capable of modifying the infection. In 9 experiments, there were 162 treated chicks: 79 per cent developed patent infections; there were 98 controls of which 93 per cent showed parasitemia. Development of infection was slower in the treated group.

8. Concentrated extracts of bovine blood serum and plasma did not modify the infection.

9. An extract of chicken blood cells, prepared in the same manner as that from bovine blood cells, produced a similar modifying effect. The activity of extracts of chicken spleen and of chicken blood cells against *P. gallinaceum* in vitro and in vivo, respectively, indicates the lack of association between anti-plasmodial effect and refractoriness to the parasite on the part of the animal furnishing the extracts.

10. The active principle in bovine blood cell extract has not been identified, but certain characteristics suggest that it either is a protein, or is closely associated with the protein components of the extract.

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# SOME DEVELOPMENTS IN INSECTICIDE DISPERSAL EQUIPMENT[1]

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Widespread use of DDT insecticides during recent years has created a need for dependable spray equipment which will perform efficiently under new conditions imposed by such factors as large-scale intensive spraying programs, the corrosive action of DDT on certain metals, and the deteriorating effect of solvents such as xylene, kerosene, and fuel oil on rubber, leather, and other composition parts.

Experimental work directed towards the improvement of insecticide dispersing equipment has included use of new materials for hand spray cans in both single- and double-tank arrangements, re-design of standard hand-shutoff valves, and the construction of new types of power-operated insecticide applicators.

Agricultural-type compressed air sprayers, commonly referred to as knapsack-type sprayers, were widely used for applying DDT sprays when the first large-scale DDT residual house spraying program was inaugurated in 1945 (Stierli, *et al.*, 1945). These were $2\frac{1}{2}$- to 4-gallon capacity, hand-pumped, galvanized iron spray-cans. Minor modifications necessary to adapt such commercial hand sprayers to DDT spraying included the use of xylol-resistant hose and gaskets, clips for holding the wand to the spray can, and a nozzle that produced a flat-fan spray pattern instead of the usual conical-shaped spray pattern. However, due to the large number of man hours required to hand pump these cans, many of the spray trucks were soon equipped with air compressors and large compressed-air tanks, and the spray cans were fitted with Schrader[2] type air valves. They could then be charged with compressed air by means of an air hose and chuck, and spraying operations were greatly facilitated.

*Aluminum Spray Can with Liquid Seal.* An aluminum spray-can utilizing a liquid seal around the filler opening was designed to overcome some of the difficulties encountered with commercial sprayers such as corrosion of the galvanized iron cans and air leakage around the rubber gasket on the main head opening. Sealing of the main gasket with liquid is accomplished by placing the filler-opening in the bottom of the can so that the liquid inside the can completely covers the opening, thereby preventing any loss of air around the gasket. An additional feature of this can, designed to eliminate the need for re-tightening of the filler-hole cover, is the oval shape of the bottom opening which permits use of a cover that seats from the inside of the tank instead of from the outside. This design eliminates a bulky head-tightening mechanism and utilizes the internal pressure to supplement the mechanical sealing pressure furnished by the exterior spring clamp. An air valve at the top of the can is the only method of introducing compressed air into the spray can; there is no hand pump.

[1] From Communicable Disease Center, Technical Development Division, Savannah, Georgia.

[2] Product of A. Schrader's Son, Brooklyn, New York.

This 4-gallon capacity can was designed for a working pressure of 60 psi and weighs $9\frac{1}{2}$ pounds when empty.

The upper view in figure 1 shows the bottom of the sprayer with oval-shaped cover and opening. The lower view of figure 1 shows the top of the spray can fitted with the air valve.

FIG. 1. Four-gallon-capacity aluminum spray can. Upper view shows internally-seated oval-shaped cover in bottom of can; lower view shows top of can with air valve and discharge line.

## CONSTANT-PRESSURE HAND SPRAYERS

A uniform dosage of DDT on the walls of houses being treated in malaria control and other residual spraying programs is desirable but seldom achieved, due to several variables encountered in the spraying operation. Important among the causes of non-uniform applications is the variable discharge rate of the spray emerging from a single-charge spray can which suffers a diminishing air pressure as the contents of the can are emptied. With a constant air pressure in the liquid tank to guarantee a steady rate of discharge, there is greater likelihood of obtaining uniform applications. For this reason constant-pressure sprayers are gaining favor for the application of DDT residual sprays; also, experience has shown that the output of insecticide per man-hour is greater with the use of constant-pressure sprayers than with the use of hand-pumped variable-pressure spray cans.

A constant-pressure sprayer as referred to here is a hand-, shoulder-, or back-

carried sprayer consisting of two separate tanks having approximately equal volumes, one tank being for compressed air and the other for liquid insecticide. An initial compressed-air charge of 80 to 200 psi pressure is placed in the air tank and introduced into the liquid tank through a pressure reducing valve set at 35 to 40 psi. The contents of the liquid tank can then be discharged at a constant pressure.

*Georgia "Regulated Pressure" Sprayer.* Several hundred constant-pressure sprayers utilizing surplus Army Air Forces oxygen cylinders were built and used by personnel of the Georgia State malaria-control program during 1947 and 1948 (Lenert and Legwen, 1948). These stainless-steel oxygen tanks, 5 inches in diameter by $23\frac{1}{4}$ inches long with a capacity of approximately $2\frac{1}{6}$ gallons, were used for both the air

FIG. 2. At left, $2\frac{1}{6}$-gallon capacity constant-pressure sprayer developed by State of Georgia CDC personnel. At right, 3-gallon capacity constant-pressure sprayer developed by U. S. Army, Corps of Engineers.

and liquid compartments on the regulated-pressure sprayers. Two cylinders comprising a sprayer were placed on end, side by side, attached to a wooden base and equipped with a carrying handle and shoulder strap. Standard wands, nozzles, and shutoff valves were used. Total weight of the unit was approximately 18 pounds when empty.

The Georgia sprayer is shown at the left in figure 2. The sprayer shown at the right is the constant pressure pack-board sprayer developed in 1945 by the U. S. Army, Corps of Engineers.

*Constant-Pressure Sprayer of Stainless Steel.* To provide a more compact constant-pressure sprayer for hand-spraying operations, the unit shown in figure 3 was made by placing a surplus aircraft-type oxygen cylinder concentrically inside of a 4-gallon capacity stainless-steel Lofstrand[3] spray can. This model was designed for use with an external source of high-pressure air ranging from 100 to 200 psi. The hand pump

[3] Product of the Lofstrand Company, Rockville, Maryland.

in the Lofstrand can was removed, but the filler neck and other fittings were utilized in their original positions.

The inner cylinder serves as the compressed-air compartment, and the liquid compartment comprises the remaining space between the two concentric cylinders. Liquid capacity of the sprayer is nearly $2\frac{1}{2}$ gallons and the size ratio between the air and liquid compartments requires an initial air charge of 90 psi in order to discharge

FIG. 3. Two and one-half-gallon capacity constant-pressure sprayer with compressed air tank inside of liquid tank.

all of the liquid at a constant pressure of 40 psi. Weight of this model is approximately 17 pounds when empty.

*Constant-Pressure Sprayer of Aluminum.* In order to get away from the use of war surplus equipment, which is relatively inexpensive as surplus material but very expensive as new stock, a constant-pressure sprayer has been designed and built using aluminum tubing which is commercially available at a marketable price.

A preliminary model of this sprayer consisting of a $5\frac{1}{2}$-inch diameter aluminum tube placed concentrically inside of an 8-inch diameter tube is shown in figure 4. The

cylinder shown at the left in this figure illustrates the method of welding the inner tube to the end pieces prior to welding the larger tubing to the ends.

On this aluminum sprayer it was necessary to use the inner compartment as the liquid tank with the annular space between the tubes serving as the high-pressure air chamber. Liquid capacity of the tank is two gallons, and the size ratio of the tubes is such that an initial charge of 80 psi in the air compartment will discharge the liquid contents at a constant pressure of 40 psi. The sprayer is 21 inches long and weighs 17 pounds when empty.

FIG. 4. Two-gallon-capacity constant-pressure sprayer (at right) made of aluminum; inside tank is shown at left prior to fitting of outer tube.

## MISCELLANEOUS EQUIPMENT

*Sprayer Shutoff Valves.* In spraying the interiors of houses a positive-acting shutoff valve is a desirable part of hand-spraying equipment in order to prevent spray from streaking the walls and dripping onto floors. Figure 5 illustrates a type of shutoff valve that has a quick action and prevents leakage around the release pin. This is a modification of the Lofstrand pistol-grip type of shutoff valve, the main alteration being the inclusion of a hand lever to replace the single-finger trigger-type release on the original valve. All parts of the shutoff valve are metal, including the shutoff mechanism which employs a stainless-steel ball on a lead seat. A flexible-metal bellows seals the valve against leakage around the release pin.

*Wands.* Light-weight spray wands of any desired length have been constructed using aluminum tubing and standard automotive compression fittings which require no soldering or welding to obtain a tight connection.

*Nozzles.* Nozzle bodies and caps of aluminum alloy have been made to replace the brass nozzle bodies which hold the fan-pattern nozzle tips. Reduced weight on the end of the wand lessens the fatigue of operators. Stainless-steel fan-pattern tips are

used with the aluminum alloy nozzle bodies to give longer service than could be expected from brass tips.

## POWER SPRAYERS

*Institution Sprayer.* A small, light-weight, caster-mounted power sprayer suitable for applying insecticides and other sprays to hospitals, hotels, warehouses and similar

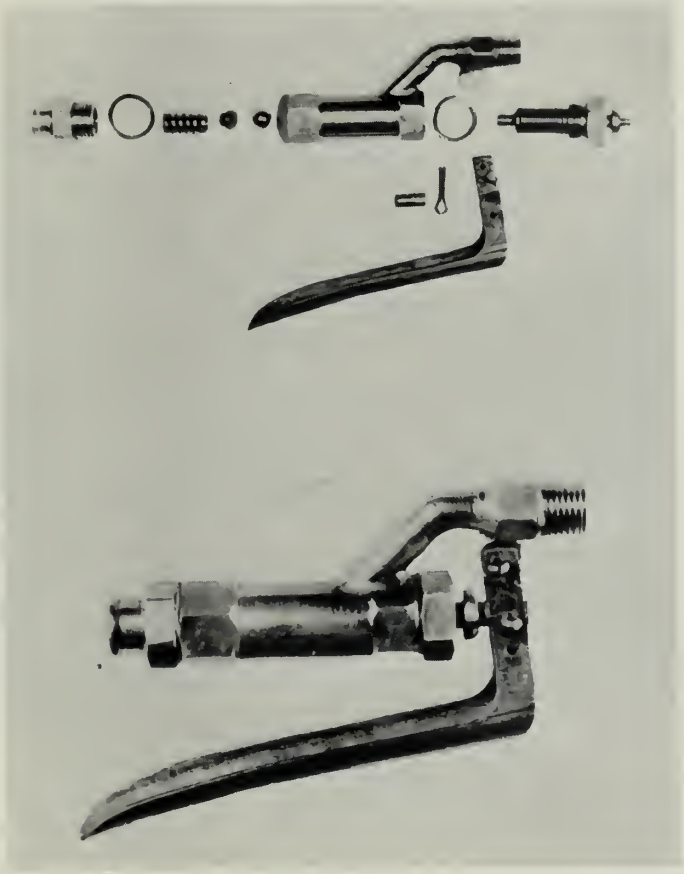

FIG. 5. Disassembled view (upper) and assembled view (lower) of modified Lofstrand shutoff valve with steel-ball shutoff and flexible-metal bellows seal.

buildings is shown in figure 6. Spray pressure in this machine is developed by a small motor-driven gear pump which draws liquid insecticide from the 12-gallon tank for delivery to two spray-wands at the ends of two 50-foot hoses. Agitation of the insecticide in the tank is accomplished by recirculating part of the spray through a by-pass system. The spur-gear pump is equipped with special shafts and bearings suitable for use in circulating non-lubricating liquids without using petroleum or other type lubricants. Either a $\frac{1}{8}$-horsepower electric motor or a $\frac{1}{2}$ horsepower gasoline engine can be used on this sprayer for driving the pump.

The aluminum truck is 20 inches wide by 28 inches long and the tank is made of aluminum with a hose reel on either side, each one suitable for holding 50 feet of lightweight spray hose. The entire unit weighs 82 pounds when equipped with the electric motor and 93 pounds with the gasoline engine attached.

*Multi-Purpose Insecticide-Dispersing Machine.* The multi-purpose insecticide machine shown in figure 7 was designed to provide a single piece of equipment that would be capable of applying the most effective type of insecticidal treatment to any one of a variety of problems which might occur in a community. The multi-purpose disperser can be used for making outdoor applications of mist or dust, or a combination of mist and dust. A residual-spraying hose and wand is attached for indoor or outdoor residual-spraying needs. It is believed that small municipalities might find such a multi-purpose machine an economical answer to their various insecticide-dispersing problems.

FIG. 6. Institution sprayer, utilizing aluminum truck and tank, showing electric-motor-powered arrangement.

The machine is basically a Gustafson[4] "Choke-Proof" rotating dust hopper and paddle-type fan. These parts have been mounted on a base 34 inches wide and 42 inches long with a 6-horsepower gasoline engine to drive the 16-inch-diameter fan and furnish power for turning the dust hopper. A rotating spiral-spring inside the hopper carries insecticidal dusts to the fan-inlet opening where they are picked up by the air stream and discharged through a 4-inch-diameter flexible metal hose.

A small spur gear pump delivers liquid insecticide under pressure to the end of the

[4] Product of Gustafson Manufacturing Company, Corpus Christi, Texas.

air nozzle where it is sprayed into the air blast and distributed as a mist. Detached 55-gallon drums serve as the insecticide tanks.

A 15-gallon-per-minute transfer pump which can be engaged at will serves to transfer liquid insecticides from containers on the ground to the drums on the truck.

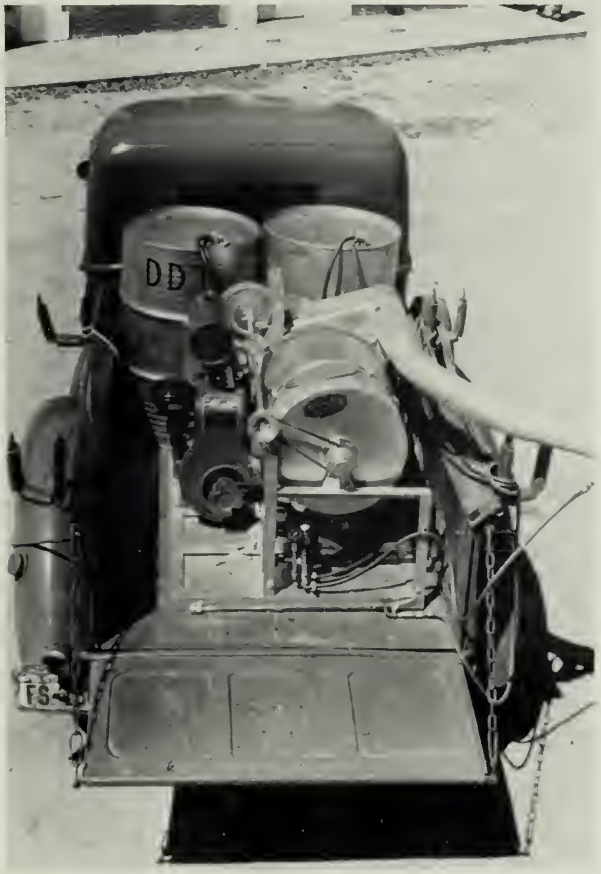


FIG. 7. Multi-purpose insecticide disperser shown mounted on ½-ton pick-up truck with two 55-gallon drums for liquid tanks.

Size of the machine is such that it will easily fit onto a ½-ton pick-up truck with sufficient room left for two 55-gallon drums.

*Graphitar*[5] *Bearing Pump.* Excessive wear of ordinary shafts and bearings in pumps used to circulate DDT-xylene emulsions has resulted in the construction of a 15-gpm spur-gear pump having monel shafts and Graphitar (carbon and graphite) bearings. This combination has been found to be very satisfactory for use in pumping DDT emulsions, as the circulant serves to lubricate the shafts and bearings without requiring a petroleum lubricant.

[5] Made by The United States Graphite Company, Saginaw, Michigan.

SUMMARY AND CONCLUSIONS

A discussion of the improvements made in hand-spray equipment for applying DDT and other new insecticides points out that the use of an outside source of compressed air permits more efficient use of personnel in residual spray programs. Descriptions are given of new constant-pressure sprayers and an improved shut-off valve. Use of light-weight wands and nozzles lessens fatigue for operators, and the use of corrosion-resistant metals in newly designed spray cans is expected to increase their useful life and reduce the need for repairs.

A description is given of a light-weight, caster-mounted power sprayer that is either gas-engine or electric-motor driven. This machine is intended for use in hospitals, hotels, warehouses, and similar structures.

A multi-purpose insecticide-dispersing machine suitable for applying spray, mist and dust or a combination of mist and dust, has been designed to fit onto a $\frac{1}{2}$-ton pick-up truck.

A spur gear pump has been made suitable for circulating DDT-xylene emulsions by use of monel shafts and Graphitar bearings.

REFERENCES

1. Stierli, H., Simmons, S. W., and Tarzwell, C. M. 1945. Operational procedures and equipment used in the practical application of DDT as a residual house spray. Public Health Reports, Supplement No. 186.

2. Lenert, L. G. and Legwen, W. A. 1948. A regulated pressure sprayer for malaria control operations. CDC Bulletin, April-May-June, 1948; also, A review of current Georgia malaria control operations. J. Natl. Mal. Soc., 7(3): 187–197 (1948).

# 31ST ANNUAL MEETING OF THE NATIONAL MALARIA SOCIETY

*Held Conjointly with the American Society of Tropical Medicine, the American Academy of Tropical Medicine, and the American Society of Parasitologists*

MINUTES—1948

*Officers*

President—Dr. E. Harold Hinman, Norman, Oklahoma
President-Elect—Dr. Wendell Gingrich, Galveston, Texas
Vice President—Mr. Nelson H. Rector, Atlanta, Georgia
Secretary-Treasurer—Dr. Martin D. Young, Columbia, S. C.

Directors:

Mr. L. M. Clarkson, Atlanta, Georgia
Mr. W. H. W. Komp, College Park, Md.
Dr. E. L. Bishop, Chattanooga, Tenn.

*Monday, December 6, 1948*

The National Malaria Society convened for its 31st annual meeting in the Roosevelt Hotel, New Orleans, Louisiana, at 9:00 a.m. with President E. Harold Hinman presiding. Dr. Hinman announced the election of the following new officers for 1949:

President—Dr. Wendell Gingrich
President-Elect—Dr. Paul F. Russell
Vice President—Dr. Ernest Carroll Faust
Director (for 3-year term)—Mr. H. W. Van Hovenberg

and appointed temporary Committees on Auditing and Resolutions. At the conclusion of the President's Address, nine papers were read and two presented by title. The session adjourned at 12:00 noon.

*Tuesday, December 7, 1948*

The Society reconvened at 9:00 a.m. for a conjoint meeting with the American Society of Tropical Medicine and the American Society of Parasitologists during which the Presidents of each of the three societies presided. A program of nine papers was presented. The meeting adjourned at 11:50 a.m.

Beginning at 2:00 p.m., the National Malaria Society held a panel discussion on "Large Scale Insecticidal Application and Machinery." Dr. E. L. Bishop acted as Moderator. The topics (1) airplane application, (2) ground applications with power equipment, and (3) techniques for the recovery and measurement of dispersed insecticides were presented both by formal and informal discussions. The session adjourned at 4:50 p.m.

*Wednesday, December 8, 1948*

The scientific session reconvened at 10:18 a.m. Eight papers were read and five presented by title. Dr. Hinman introduced the incoming President, Dr. Wendell Gingrich, who adjourned the annual meeting *sine die* at 12:05 p.m.

The business meeting was held at 9:00 a.m. with President Hinman presiding. The minutes of the 1947 annual meeting in Atlanta, Georgia, were approved as published in the March, 1948, issue of the *Journal of the National Malaria Society*.

The report of the meetings of the Board of Directors during 1948 was read.

The Secretary-Treasurer reported as follows:

From the 1947 roster of 554 active members, three (Dr. T. J. LeBlanc, Dr. R. P. Strong, and Major Roy King) have been lost by death; eleven have resigned; and thirty-nine have been dropped because of delinquency in dues. During the year seventy-one new members have been elected, making an active membership of 572 and representing a gain of eighteen members; of these, 457 are in good standing as of November 30, 1948.

The status of the treasury, at the close of business of November 30, 1948, was:

| | |
|---|---|
| Balance reported November 29, 1947 | $4,484.33 |
| Receipts from delinquent, current, and advance dues, subscriptions, advertising, sales of back issues, interest, et cetera | 4,802.14 |
| Grant from the American Foundation for Tropical Medicine | 1,000.00 |
| Total | $10,286.47 |
| Less Expenditures before paying for the 4th issue of the 1948 Journal, but including the cost of printing the 3rd and 4th numbers of the 1947 Journal | 4,148.61 |
| Balance on Hand | $6,137.86 |

Of the balance on hand, November 30, 1948, $5,699.39 is in the publication account, and $438.47 is in the operating account.

Assets estimated for the year 1948, ending December 31, including the above cash balance, total $6,459.81; estimated liabilities are $1,298.93, which leave the estimated net resources available at the end of the year to be $5,160.88.

This report was accepted by the Society.

Mr. Nelson Rector, Chairman of the Committee on Auditing, stated that the books and accounts of the Secretary-Treasurer had been examined and found in order; he recommended that honoraria be granted in the amount of $200.00 to the Secretary's stenographer and $25.00 to the Editor's stenographer. The Society adopted the report and recommendations of the Committee.

Speaking for the Committee on Medical Research, Dr. G. Robert Coatney presented a progress report which was accepted on motion.

Since Dr. E. C. Faust was unable to attend the business session, short reports of the Committee on Statistics and the Representative to the Council of the American Association for the Advancement of Science were read by the Secretary and subsequently adopted.

Dr. Paul F. Russell, reporting for the Policy Committee of which he served as Chairman, said that after careful study it seemed desirable to seek a closer affiliation

with the American Society of Tropical Medicine and the American Academy of Tropical Medicine but that an amalgamation did not appear advisable at the present time. Although the Committee favored a revision in the name of the Society to show a broadening interest in the field of arthropod-borne diseases, no specific recommendation was made due to the fact there was no agreement upon the choice of a new name.

There was much discussion by the members relative to this report. Dr. Russell moved that the incoming President appoint a Committee on Policy to further consider broadening the base of the Society and recommended that this committee also consider federation with the American Society of Tropical Medicine and the American Academy of Tropical Medicine. The motion passed.

Dr. Hinman gave a report on the Fourth International Congresses on Tropical Medicine and Malaria, pointing out the important part the National Malaria Society's members had contributed.

For the Committee on Resolutions, Dr. Griffith Quinby introduced resolutions expressing the Society's regret upon the death of active members, Dr. T. J. LeBlanc, Dr. R. P. Strong, and Major Roy King, and inactive Charter Members, Surgeon General Rupert Blue, USPHS (Ret.), and Rear Admiral E. R. Stitt, MC, USN (Ret.), instructing the Secretary to transmit to each of the bereaved families an appropriate expression of sympathy. Other resolutions expressed the appreciation of the Society to the Committee on Local Arrangements of which Dr. W. A. Sodeman was Chairman; to the Committee in Charge of Arrangements for Ladies' Entertainment, Mrs. J. S. D'Antoni, Chairwoman; to the Schools of Medicine of Tulane University and Louisiana State University, the host organizations for their generous efforts which contributed greatly in making the meetings a success; to the management of the Roosevelt Hotel for the adequate facilities provided; and to Editor F. L. Knowles and to Secretary M. D. Young for their work on behalf of the Society.

It was further resolved that the Society gratefully acknowledge its appreciation to the American Foundation for Tropical Medicine for the generous contribution of $1,000.00 which made possible the publication of many papers which otherwise would have been deferred. The National Malaria Society accepted all of the proposed resolutions.

Mr. W. H. W. Komp motioned that the By-Laws be amended to show an allocation of $3.00 of each member's dues for a subscription to the Journal and that the first sentences in paragraphs b and c of By-Law 4 be changed to read $3.00 instead of $2.00. This motion was carried.

The Society voted favorably upon the motion by Dr. G. Robert Coatney that a blank nomination ballot for officers be mailed to the membership with the statement of annual dues for 1949.

The business meeting adjourned at 10:17 a.m.

# NEW MEMBERS OF THE NATIONAL MALARIA SOCIETY

1948

(Additions to the Lists Published in the Journal:
**4**(4): 351–64—December, 1945
**7**(1): 79–83—March, 1948)

ADAMS, Dr. P. C. G., African Medical Training School, Lusaka, Northern Rhodesia, Central Africa.

AGUILAR, Dr. Francisco J., Colonia 25 de Junio, 3a Av. 107, Guatemala, Guatemala.

AKINS, Mr. Harvey, USPHS, Laboratory of Tropical Diseases, 874 Union Avenue, Memphis, Tenn.

ANDERSON, Dr. Richmond Karl, c/o Rockefeller Foundation, 49 West 49th Street, New York 20, N. Y.

ANDERSON, Mr. Thomas P., 1218 Sherwood Road, Columbia, S. C.

ARMSTRONG, Mr. Robert L., Carlisle Road, Westford, Mass.

*BERTI, Dr. Arturo Luis, Division de Malariologia, Maracay, Aragua, Venezuela.

BINDARI, Dr. Abdel Aziz, 15 Rashdan Str., Orman, Giza, Cairo, Egypt.

BOYD, Dr. George Hugh, Baldwin Hall, Univ. Georgia, Athens, Ga.

CALDEIRA, Dr. Oscar Versiani, Rua dos Timbiras, 2344, Belo Horizonte, Minas Gerais, Brazil.

CARBEL, Dr. Thomas J., Av. Mitre 956, Tucuman, Argentina.

CASTLEMAN, Mr. Frederick W., 301 S. Bonham St., Columbia, S. C.

CHANCO, Dr. Pedro P., Jr., 522 San Antonio St., Paco, Manila, Philippines.

CHANG, Dr. Teh-Ling, Malaria Laboratory, National Institute of Health, Nanking, China.

COFFEY, Mr. Joseph H., USPHS Virus Laboratory, Box 436, Route 3, Montgomery, Ala.

COLL, Dr. Hector A., Avenida Mitre 956, Tucuman, Argentina.

CONWELL, Dr. Donald P., D.C.C.M. Wema, via Coquilhatville, Congo Belge, W. C. Africa.

DAGGY, Dr. Richard H., Arabian American Oil Co., Dhahran, Saudi, Arabia.

DAVIDSON, Mr. Charles M., 216 Old Post Office, TVA, Chattanooga, Tenn.

*DE AZEVEDO, Dr. Joao Fraga, Instituto de Medicina Tropical, Tr. do Porto Franco, Lisbon, Portugal.

DE CAIRES, Dr. P. F., Box 199, Georgetown, Demerara, British Guiana.

DE GUZMAN, Dr. Milagros S., 75 M. Salvador, San Juan, Rizal, Philippines.

DOBROVOLNY, Dr. Charles G., APO 839, c/o Postmaster, New Orleans, La.

DOWLING, Dr. M. A. C., c/o Medical and Health Dept., Mauritius.

FARID, Dr. Mohyeddin Ahmed, 28th Riad Pasha Street, Helwan, Egypt.

FERREIRA, Dr. Mario de Oliviera, Rua Duarte Schutel 22, Florianopolis, Estado de Santa Catharina, Brazil.

*GALICH, Dr. Luis F., Direccion General de Sanidad Publica, Guatemala, Guatemala.

* Membership effective 1949.

GAMMONS, Mr. John Gray, 2911 S. Dinwiddie St., Apt. C-1, Fairlington, Arlington, Va.

GEIGY, Dr. Johann Rudolf, Swiss Tropical Institute, Socinstrasse 57, Basle, Switzerland.

GENTHER, Mrs. Clara Sesler, Institute of Med. Research, Christ Hospital, Cincinnati 19, Ohio.

GILMORE, Mr. William H., 519 Dexter Avenue, Montgomery, Ala.

GLOVER, Mr. Nathaniel G., P. O. Box 931, Tuscaloosa, Ala.

*HARTMAN, Dr. Ernest, Chicago Medical School, 710 S. Wolcott Ave., Chicago 12, Ill.

*HEIDT, Mr. James H., P. O. Box 274, Dania, Florida.

HILL, Mr. Samuel O., 3rd Medical Army Lab., Fort McPherson, Ga.

JULIANO, Dr. Serafin J., 43 Dapitan Street, Mesa Hgts., Quezon City, Philippines.

KAN, Dr. Huai-Chieh, Leung Kwong Baptist Hospital, Tungshan, Canton, China.

KESSEL, Dr. John F., School of Med. Univ. Southern Calif., Los Angeles, Calif.

KINCAID, Mr. C. S., 403 Jackson Street, Paris, Tenn.

LEACH, Dr. Charles Nelson, 49 W. 49th Street, New York 20, N. Y.

LEWERT, Dr. Robert M., Dept. Bacteriology, Univ. Chicago, Chicago 37, Ill.

LOPP, Mr. Oscar V., State Dept. Health, Montgomery 4, Ala.

MAGOON, Dr. Estus H., P. O. Box 377, Coconut Grove Sta., Miami, Fla.

MAIER, Dr. John, Apartado 646, Maracay, Aragua, Venezuela.

MAKARI, Dr. J. G., Trans-Arabian Pipe Line Co., P. O. Box 1348, Beirut, Lebanon.

MARKOS, Dr. Bazil George, CDCA, 2180 Milvia St., Berkeley, Calif.

MORALES C., Sr. Ing. Salvador, Jordaens 3, Col. Tolteca, Mixoac, Mexico, D. F., Mexico.

MURTHY, Mr. N. Krishna, 161 Vanivilas Road, Basavanagudi P. O., Bangalore, India.

NEILL, Mr. Louie Cecil, Clinical Lab., VA Hospital, Montgomery, Ala.

NORMAN, Mr. James Lee, Zoology Dept., Univ. Oklahoma, Norman, Okla.

OLIVER-GONZALEZ, Dr. Jose, School of Tropical Med., San Juan, Puerto Rico.

PECKHAM, Mr. Richard S., APO 839-F, c/o Postmaster, New Orleans, La.

PHILEN, Mr. E. A., Health & Safety Div., TVA, Wilson Dam, Ala.

POWER, Mr. A. E., Box 716, Summerville, S. C.

PROSKE, Mr. Herman O., House 61, Village I, Sheffield, Ala.

ROGERS, Dr. David Leigh, Federal Correctional Institution, Seagoville, Texas.

SEATON, Mr. Harold B., Drawer 1586, Wilson Dam, Ala.

*SELF, Dr. J. Teague, Dept. Zoological Sciences, Univ. Oklahoma, Norman, Okla.

SERGENT, Dr. Edmond, Institut Pasteur D'Algerie, Algiers, Algeria.

*SHATTUCK, Dr. George C., Harvard Medical School, Boston, Mass.

SHELL, Mr. William E., Jr., 3300 St. Charles Ave., Apt. 31, New Orleans, La.

*SINGH, Lt. Col. Jaswant, 22, Alipore Road, Delhi, India.

SMITH, Dr. Hugh Hollingsworth, Rockefeller Foundation, 49 W. 49th Street, New York 20, N. Y.

SOBTI, Mr. S. K., Kamptee Road, Nagpur, Central Provinces, India.

* Membership effective 1949.

STERLING, Mr. Clarence I., Jr., 8904 Sudbury Road, Silver Spring, Md.
*STIERLI, Mr. Harry, CDCA, P. O. Box 769, Savannah, Ga.
STRANDTMANN, Dr. Russell W., Dept. Biology, Texas Technological College, Lubbock, Texas.
SUNDARAM, Dr. R., State District Health Officer, Public Health Dept., Trivandrum, Travancore State, India.
SUNDARARAMAN, Mr. S., Johns Hopkins Sch. Hygiene, 615 N. Wolfe Street, Baltimore 5, Md.
TEJEDA FONSECA, Dr. Carlos, 16 Calle Poiente No. 39, Guatemala, Guatemala.
TOMLINSON, Dr. Thomas H., Jr., USPHS, Lab. Tropical Diseases, 874 Union Ave., Memphis, Tenn.
†VAN HOOF, Dr. Lucien, Institut de Medecine Tropicale, Prince Leopold, 155 Rue Nationale, Antwerp, Belgium.
VANN, Mr. Irvin B., Sr., Box 38, Edison, Ga.
VONDERLEHR, Dr. Raymond A., CDC, 605 Volunteer Bldg., Atlanta, 3, Ga.
WELCH, Miss Sarah F., CDC, 605 Volunteer Bldg., Atlanta 3, Ga.
WILSON, Mr. William F., Div. Health & Safety, TVA, Wilson Dam, Ala.
WOLCOTT, Dr. Gordon B., USPHS, P. O. Box 717, Columbia, S. C.
WRIGHT, Dr. Willard H., Lab. of Tropical Diseases, National Inst. Health, Bethesda 14, Md.
YEAGER, Dr. Clark H., 13 Midvale Road, Baltimore, 10, Md.
YOGORE, Dr. Mariano G., Jr., Inst. Hygiene, Univ. Philippines, Manila, Philippines.

* Membership effective 1949.
† Deceased.